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Growth of gold nanoclusters and nanocrystals induced by lysozyme protein in thin film conformation

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ABSTRACT

Structures and growth behavior of gold nanoclusters and nanocrystals have been explored on thin films of globular protein lysozyme by using UV–vis and photoluminescence spectroscopy, X-ray diffraction (XRD) and atomic force microscopy (AFM). A simple and one-step environment friendly method has been used to grow nanocrystals on protein surface from $HAuCl_4$ solution. It has been found that if different interaction times are provided between lysozyme films and $HAuCl_4$ solution, then initially formed tiny gold nanoclusters on protein surface transform into nanocrystals with the passage of time. XRD analysis shows the formation of faced-centered cubic lattice along (1 1 1) crystalline direction and AFM images confirm the presence of circular, rod-like, triangular and hexagonal crystal structures. Langmuir-like growth behavior has been identified for both the gold nanoclusters and nanocrystals formation induced by the lysozyme films, however, nanocrystal growth is relatively slower than nanocluster.

1. Introduction

In recent decades improvement on nanoscience and nanotechnology have demonstrated that nanostructured materials like nanoparticles, nanorods, nanoplates, etc. have potential applications in the field of biology, medicine, data storage, catalysis, sensors, etc. [1–3]. The interdisciplinary work between nanoscience and biology especially biotechnology have created a new frontier in which multifunctional smart structures could be designed which have potential applications [4]. Current efforts in this area is to produce biocompatible nanomaterials which can be conjugated to peptides, proteins, DNA, etc. [5–11] to open a new platform for integrating biology and synthetic materials. In this regards recently many important works have been reported on bioconjugation between biomolecules and nanoparticles where bioconjugation with nanoparticles have been possible by using some intermediate protective agents of the nanoparticles or by using linking agents [12–14]. However, bioconjugation is usually a chemical strategy to form a stable covalent linkage between two molecules and at least one of them is a biomolecule. Biomacromolecules like proteins are one of the best candidates for bioconjugation and for the synthesis of different nanostructures of varying shape and size due to their self-reducing properties.

Among the board range of nano materials, gold in particular have gained huge attention due to their unique optical properties, wide usability in biological systems, biolabeling, high chemical stability, etc. [15–19]. Depending upon the shape, size, agglomeration, local environment, etc. optical behavior of gold nanoparticles modifies. The size and shape dependent optical properties of gold nanoparticles is due to the collective oscillation of conduction band electrons upon irradiation with the light of appropriate frequency which is called Plasmon frequency [20]. Shape and size variation of nanoparticles are possible by using a wide range of chemical, physical and biological techniques [15,21–23]. However, in chemical methods, involvement of different precursors, reducing agents, capping agents, etc. make it hazardous and incompatible to humans and environments. Therefore, new steps have been taken toward biosynthesis of gold nanoparticles by using bacteria, yeast, proteins, plant and flower extracts, etc. [24–27]. Nanomaterials synthesized by these processes show the same characteristic properties as synthesized by others chemical and physical methods [28]. Proteins like bovine serum albumin (BSA), lysozyme, pepsin, etc. have been found to be very much useful for different nanostructures formation [12,29–33]. Biosynthesis of gold, silver and their alloy nanoparticles using bovine serum albumin (BSA) have been reported [29–32]. Synthesis of gold nanoparticles in aqueous medium through chemical reduction of chloroauric acid in the presence of proteins has been reported. In solution, growth behavior of nanocrystals has also been studied and it has been found that

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the nanoparticles mostly follow the diffusion-limited 'Ostwald ripening' growth [34,35], where the average particle diameter has a cube-root dependence on the particle forming time. However, non-Ostwald-ripening growth nature has also been reported for both metal and semiconductor nanoparticles [36,37]. Although several works have been reported on the formation of metallic nanoparticles or nanocrystals using proteins in bulk solution but there are very few on protein thin film induced nanoparticle growth.

In this article, we have studied on the structures and growth of gold nanoclusters and nanocrystals formation on the lysozyme thin films in the presence of $HAuCl_4$ aqueous solution. UV–vis absorption and photoluminescence emission spectroscopy confirms the formation of gold nanoclusters and nanocrystals. Growth direction of the crystal plane has been investigated through X-ray diffraction (XRD) studies. Surface morphology of the films and the different shapes and sizes of gold nanocrystals have been obtained through atomic force microscopy (AFM). Langmuir-like growth behavior has been identified for the lysozyme film induced gold nanoclusters and nanocrystals formation.

2. Experimental

Lysozyme (catalog No. 62971) and Gold (III) chloride trihydrate ($HAuCl_4$) (catalog No. 520918) were purchased from Sigma. Quartz slides used for the preparation of protein thin films were purchased from Alfa Aesar. Ultrapure Milli-Q water was used throughout the experiment. Lysozyme solution of 15 mg/ml was prepared by dissolving required amount of lysozyme in Milli-Q water and the concentration of $HAuCl_4$ was taken as 0.5 mM.

Quartz substrates were cleaned by treated with a mixture of ammonium hydroxide (NH_4OH, Merck, 30%), hydrogen peroxide (H_2O_2, Merck, 30%) and Milli-Q water (H_2O:NH_4OH:H_2O_2 = 2:1:1, by volume) for 5–10 min at 100 °C followed by drying at normal room temperature (25 °C). Lysozyme thin films were prepared on quartz substrates by spreading the desire amount of lysozyme solution on substrates and then spin coated the films by using Apex spin-coater (Spin XNG-P2) at a speed of 1000 rpm. After spin coating, the films were left overnight for drying at normal room temperature. It took nearly 12 h for complete drying of the films and then dipping were done within 3–4 h. Dipping experiments were done by slowly putting the lysozyme film coated quartz substrates inside the 0.5 mM $HAuCl_4$ solution and keeping undisturbed inside solution for different time periods. Due to hydration few loosely attached molecules may come out in water from the film surface. The respective dipping time was set as 5 min (sample-1), 15 min (sample-2), 30 min (sample-3), 45 min (sample-4) and 60 min (sample-5) respectively. After taking out from the solution all films were kept at normal room temperature for drying and kept in dark before characterizations.

UV–vis spectra were taken using Shimadzu UV-1800 UV–vis spectrophotometer and the photoluminescence emission spectra were recorded by using fluorescence spectrometer (Cary Eclipse) [38]. X-ray diffraction (XRD) measurements were carried out using D8 Advanced, Bruker, AXS (XRD) setup [39]. The diffractometer consists of a Cu source (sealed tube) followed by a Göbel mirror to select and enhance Cu $K\alpha$ radiation (λ = 1.54 Å). Scattered beam was detected using NaI scintillation (point) detector. Morphological information of lysozyme films before and after interaction with $HAuCl_4$ were obtained through an atomic force microscope (NTEGRA Prima, NT-MDT Technology) in semi-contact mode using silicon cantilever having spring constant of ~11.3 N/m [36]. Scans were performed over several portions of the films for different scan areas.

3. Results and discussion

3.1. UV–vis spectroscopy

UV–vis absorption spectra obtained from the lysozyme films before and after interaction with $HAuCl_4$ solution are shown in Fig. 1a. UV–vis absorption technique is one of the simplest and convenient ways to confirm the formation of nanoclusters and nanocrystals. Pure lysozyme film shows only absorption peak at ≈271 nm which is the characteristic absorption of lysozyme molecules. When the lysozyme thin films make contact with $HAuCl_4$ solution for different time periods as mentioned in the experimental section, two peaks evolve at ≈224 nm and ≈320 nm respectively in the absorption spectra. The peak at ≈224 nm is due to the chloroaurate ions ($AuCl_4^-$) as seen by Kunio et al. [40] and the band at ≈320 nm is attributed to gold nanoclusters as also seen by Mosseri et al. [41]. Lysozyme is a well known water soluble protein molecule and has potential for synthesizing different nanomaterials in solution in the presence or absence of different reducing agents. Any extra reducing agent is not used in this work as lysozyme itself acts as a reducing reagent. Thus, UV–vis study confirms the formation of gold nanoclusters from chloroaurate ions on protein surface. If those films are kept in dry condition for ≈30 days, then UV–vis spectra obtained from the films show different information of nanocrystals formation which is shown in Fig. 1b. Peaks obtained at ≈224 and 320 nm nearly vanishes and a new peak is observed nearly at ≈520 nm which is the characteristic localized surface plasmon resonance (LSPR) peak of gold nanoparticles

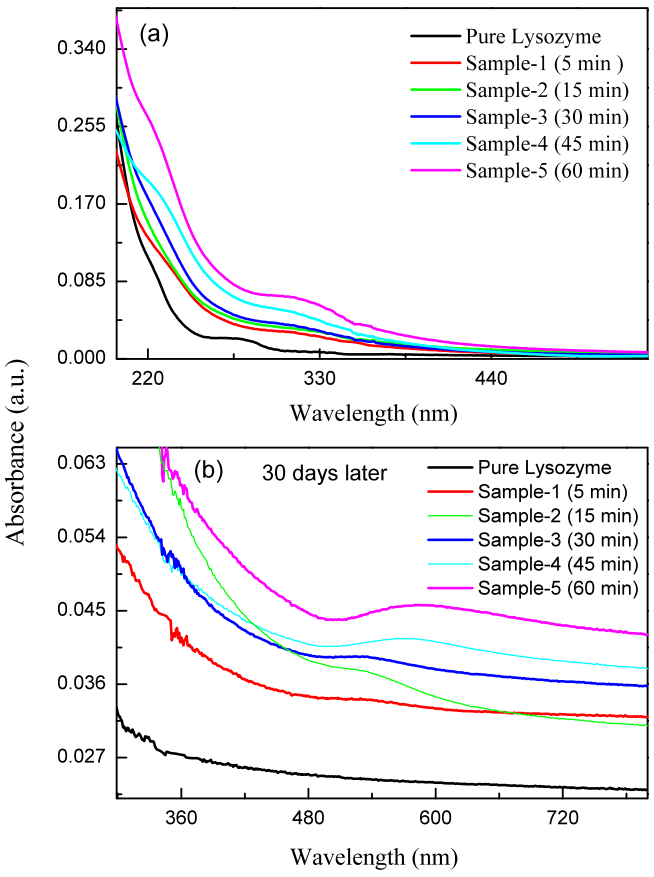

Fig. 1. (a) UV–vis spectra of pure lysozyme film and lysozyme film after interaction with $HAuCl_4$ aqueous solution for specific time interval of 5 min (sample-1), 15 min (sample-2), 30 min (sample-3), 45 min (sample-4) and 60 min (sample-5). (b) UV–vis spectra taken from the same samples (i.e., from pure lysozyme and sample-1 to sample-5) after 30 days.