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Protein interactions with silver nanoparticles: Green synthesis, and biophysical approach

Nada Shaeel AL-Thabaiti, Maqsood Ahmad Malik, Zaheer Khan*

Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah, 21589, Saudi Arabia

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ABSTRACT

Silver nanoparticles (AgNPs) with an average particle size of 20 nm were synthesized by using aromatic amino acid fluorescence active, tryptophan as a reducing agent. This study aims to investigate the interaction between Bovine Serum Albumin (BSA) and AgNPs as a function of particle size and shape. UV–vis analysis implies the formation of the ground state complex between BSA and AgNPs through electrostatic interactions. The fluorescence spectra indicated that the AgNPs have a potent ability to quench the intrinsic fluorescence of BSA by static quenching mechanisms. The different parameters (the apparent association constant (K_{app} = 2.6 × 10^4 mol^{-1} dm^3), Stern–Volmer quenching constant (K_{SV} = 3.5 × 10^4 mol^{-1} dm^3), number of binding sites (n = 1.3) and bimolecular rate constant of the quenching reaction (k_q = 6.1 × 10^{12} mol^{-1} dm^3 s^{-1})) were calculated by using the UV–vis and fluorescence spectra and discussed. The indole moieties of tryptophan residues of BSA were responsible to the complex formation with AgNPs in ground and excited states via electrostatic, van der Waals, hydrogen bonding, hydrophobic and hydrophilic interactions. Adsorption of AgNPs into the core of BSA changes the tryptophan environment from hydrophobic to hydrophilic (from folding to partially folded and/or unfolded). Circular dichroism results suggested that the helicity of BSA decreased from 67.68% to 60.25% and 67.68% to 45.42% with [AgNPs] and temperature, respectively.

1. Introduction

Owing to regulate the colloidal osmotic pressure of blood, the chemistry of water soluble protein (BSA; single polypeptide chain consisting of about 582 amino acid residues, two tryptophan moieties at positions 134 and 212 as well as tyrosine and phenylalanine. At pH 5–7 it has 17 intra chain disulfide bridges and 1 sulfhydryl group) in terms of their biophysical and physicochemical properties in solution has come a long way and their importance in various fields such as interaction with drugs, nanoparticles, polymers, surfactants, etc. are unlimited [1–10]. The literature is also replete with the investigations of the use of BSA as the reductant and/or capping agent for carrying out the syntheses of advanced nano materials of silver and gold [11–14]. The information on the interaction of BSA with nanoparticles would reflect to understand the absorption and distribution of the nanoparticles and play an important role in pharmacology and pharmacodynamics. Sastry et al. used BSA as a reducing and stabilizing agent for the synthesis of mono- and bimetallic nanoparticles of gold and silver and also suggested that dual behaviors of BSA, zwitterionic surfactant and foaming nature were necessary for the synthesis bimetallic nanomaterials at the protein isoelectric point [15]. Bakshi and his coworkers reported synthesis of gold nanoparticles by using BSA protein as a reducing agent [16]. They also pointed out that the unfolded state of BSA reduced the Au^{3+} into Au^0 due to the presence of cysteine in the molecular structure of BSA and it also provides excellent capping and/or stabilization properties, which were essential for colloidal behavior of nanoparticles in water [17].

Henglein et al. in their pioneering featured article reported that the small metal particles in solution have been found to be advantageous over the water-insoluble forms because a UV–vis spectrophotometer can be used to monitor the optical

changes that accompany the surface reactions of metal and semiconductor nanoparticles with plasmon resonance lines in the visible range (solutions of nanometer) large particles are transparent and the scattering of light can be neglected [18]. On the other hand, polar nature of water-soluble globular proteins are more prone to the interactions with metal ions, nanoparticles, hormones and drugs rather than fibrous proteins [15,19,20]. Proteins and peptides, with aromatic amino acids are intrinsically fluorescent when excited with UV light. The aromatic side chain amino acids (tryptophan residues, tyrosine, and phenyl alanine), lone pair

* Corresponding author.
E-mail address: drkhanchem@yahoo.co.in (Z. Khan).

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of electrons of the nitrogen and oxygen of indole ring and hydroxyl groups, ionic species, methionyl sulfur and disulfide bonds of BSA were responsible for intrinsic fluorescence and scavengers of reactive oxygen and nitrogen species which plays an important role in oxidative stress [21,22]. Out of tyrosine and phenylalanine, tryptophan is much more fluorescent probe (relatively rare amino acid), which can be used to estimate the nature of microenvironment of the tryptophan in the BSA. Therefore, water-based method was chosen for the synthesis of bio-conjugated AgNPs by using tryptophan (BSA fluorescence active aromatic amino acid) as a reductant and to determine their interactions with water soluble BSA for their possible use as drug delivery vehicles. Conventional UV–vis and Fluorescence spectroscopic techniques were used for this purpose.

2. Experimental

2.1. Materials

Double distilled (first time from alkaline $KMnO_4$), deionized, and CO_2 free water was used as solvent throughout. Bovine Serum Albumin, tryptophan, and silver nitrate were purchased from Sigma and used with out used further purification. The stock solutions of BSA (10 mg/ml) and silver nitrate (0.01 mol dm^{-3}) were prepared in sodium acetate −acetic acid buffer of pH 5.0. All the chemicals were of analytical reagent grade. The pH of the reaction mixture containing required [AgNPs] and [BSA] was adjusted to 5.0 (close to the iso electric point of BSA).

2.2. Synthesis and characterization of AgNPs using tryptophan

Tryptophan (BSA aromatic fluorescence active amino acid) was used as a reducing in the present studies. In a typical experiment, required volume of tryptophan was added to aqueous $AgNO_3$ solution (from 1.0×10^{-5} mol dm^{-3} 10.0×10^{-5} mol dm^{-3}). As the reaction proceeds, the colorless reaction mixture turned yellow to orange, indicating the formation of nanoparticles [23,24]. UV–vis Spectrophotometer (model UV-260 Shimadzu) with 1 cm light path quartz curette was used to monitor the progress of the reaction under different experimental conditions. The transmission electron microscopy (TEM) images were obtained on a JEOL, JEM-1011; Japan, transmission electron microscope operating at 160 kV. The samples for TEM were prepared by drop-casting one drop of the prepared silver sols onto carbon-coated copper grids and then drying in air. The fluorescence spectra were measured using a Shimadzu RF5300 spectro fluorometer with rectangular quartz cell at room temperature with 1 cm quartz cell. BSA was excited at 295 nm (at this wavelength, the tryptophan emission spectrum is dominant over the weaker tyrosine and phenylalanine fluorescence) [25]. The fluorescence emission was collected from 260 to 560 nm. Circular dichroism spectra of BSA in presence of different [AgNPs] were also recorded by using a JASCO J-815 spectropolarimeter equipped with a water jacket, through which water was circulated at a desire temperature. All the CD spectra were performed in the wavelength region 200–250 nm at 1-nm intervals using a cell of 0.5 mm path-length. The scan speed was 100 nm/min and response time of 1 s for all measurements. Each spectrum was the average of 2 scans.

3. Results and discussion

3.1. Morphology and concentration of AgNPs

UV–visible spectroscopy is one of the widely used techniques for characterization of metal-nanoparticles because the shape of the spectra gives preliminary information about the morphology of metal-nanoparticles. Fig. 1 shows the UV–vis spectra of AgNPs formation as a function of time. As the reaction time increases, a peak began to develop and finally as prepared AgNPs contained a sharp surface plasmon resonance band at ca. 400 nm along with a broad shoulder at 500 nm. Further to see insight into the morphology of nanoparticles and to confirm the spectroscopic data, TEM images of AgNPs were also recorded. The particles are mono dispersed, spherical, and some irregular shaped having average diameter is ca. 20 nm (Fig. 1; inset). Interestingly, no turbid solution of AgCl or AgBr was detected in presence of NaCl or NaBr. This result indicated that essentially all Ag^+ ions were transformed to the Ag^{00} during the redox process and/or adsorbed on the surface of silver cluster, i.e., Ag_4^{2+}. Thus, we may safely conclude that Cl^- or Br^- ions could not detach the adsorbed Ag^+ from the Ag_4^{2+} [26].

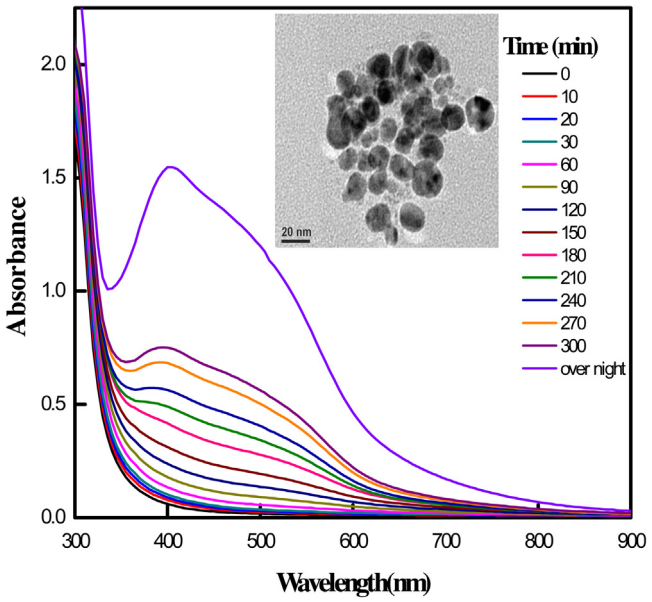

Fig. 1. UV–vis spectra of AgNPs formation as a function of time at room temperature. Inset- Transmission electron micrograph of as received AgNPs uninteracted by BSA..

In order to confirm the formation of perfect transparent silver sols and to the calculation of [AgNPs], the fulfilment of the Beer-Lambert by the resulting silver sols solution was checked. The wavelength 400 nm was chosen by monitoring the absorbance at 400 nm for different [Ag^+]. The results are depicted graphically

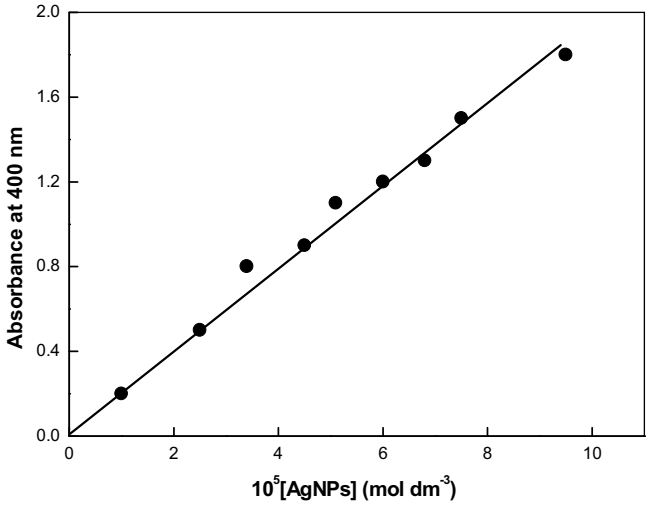

Fig. 2. The Beer-Lambert plots of AgNPs.