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Protein corona over silver nanoparticles triggers conformational change of proteins and drop in bactericidal potential of nanoparticles: Polyethylene glycol capping as preventive strategy

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ABSTRACT

Here, we demonstrated that starch-capped silver nanoparticles ($AgNP_{ST}$) with a size range of 10–15 nm could readily interact with a small protein bovine α-lactalbumin (BLA) through the formation of protein corona. We further observed that such phenomena not only caused structural change of BLA but drastic drop in the bactericidal potential of AgNP. To design a strategy towards minimizing protein adsorption and maximizing the retention of bactericidal potential of AgNP, we developed stable polyethylene glycol (PEG)-capped AgNP ($AgNP_{PEG}$) that clearly demonstrated reduced conformational changes of protein and retention of substantial bactericidal potential of $AgNP_{PEG}$, compared to $AgNP_{ST}$. Moreover, $AgNP_{PEG}$ also showed excellent hemocompatibility. A relatively larger protein bovine serum albumin (BSA) and human blood serum solution containing serum proteins were also used in this study to validate our hypotheses. Overall, our study established that protein coated AgNP losses its inherent bactericidal potential substantially; however, when functionalized with a suitable material such as PEG, it could reduce such drop in substantial amount. Moreover, it achieved improved biocompatibility in actual physiological condition that might find a better therapeutic avenue in many bacteria-mediated disorders.

1. Introduction

Biological application of any nano-material requires addressing the issues such as bioactivity and biocompatibility in biological environment. The use of nanoparticles (NP) in biological system triggers its interaction with various biomolecules including proteins. Since proteins have their unique 3-D structure and function, the little change in structure may perturb their function entirely. Proteins upon interaction with NP develop a coating known as protein corona [1,2]. The properties of protein corona on NP surface also depend on the size, shape, and surface property of NP [3]. Moreover, the formation of protein corona over NP surface not only affects the protein structure and function but also create a new identity for NP [4], which might also alter the bioactivity of the NP in the physiological condition.

Among metal NP, silver nanoparticles (AgNP) emerged as a potential antimicrobial agent due to its effectiveness against broad spectrum of bacteria [5] and fungi [6]. Moreover, its effectiveness against multi-drug resistance bacteria and low cost preparation also generated huge interest among various scientific groups to develop AgNP-based therapeutics in various bacteria-mediated diseases by varying parameters such as size [7,8], shape [9], and surface functionalization [10]. AgNP like all other metal NP also form protein corona when interacts with proteins [11] and such conjugate formation might alter the structure of proteins and bioactivity of NP. However, most of the reports regarding antibacterial potential of AgNP focussed on the role of AgNP against various strains of bacteria (both normal and antibiotic resistant), however, such reports did not address the phenomena that happens in real physiological condition where NP encounter various biomolecules such as proteins. Moreover, this interaction may alter the inherent bactericidal potential of AgNP. More specifically, the hemocompatibility of AgNP and its fate of interaction with number of blood serum proteins is a matter of concern. Therefore, it is essential to design the AgNP surface in such a manner that it could be hemocompatible and could reduce the interaction with the proteins as to high extent without losing bactericidal potential. Such strategy not only desires to minimize structural alteration of many proteins and subsequent side effects, but also maintains the bioactivity of AgNP in physiological condition.

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In order to address the problem, we studied the interaction of AgNP with a well-known small model protein bovine α-lactalbumin (BLA) to observe the alteration of its structure and function. Moreover, the effect of bactericidal potential of AgNP due to the conjugate formation with BLA was also studied. BLA is a small monomeric whey protein (Mol. Wt. is 14.2 kDa) and has been widely studied for understanding folding and unfolding mechanism under in vitro condition [12–14].

Here, we synthesized both AgNPs of equivalent average size of 10 nm size via chemical synthesis route using starch and polyethylene glycol (PEG) as capping agents. Synthesized nanoparticles were characterized using microscopy, spectroscopy, X-ray diffraction analysis, dynamic light scattering for shape and size. We further studied the interaction of AgNP with a small monomeric whey protein bovine α-lactalbumin (BLA). We monitored the conformational change of BLA upon interaction with AgNP by fluorescence as well as circular dichroism (CD) spectroscopy. The vulnerability of the protein in NP-protein conjugate towards protease attack was also examined. The effect on bactericidal activity of AgNP due to the formation of NP-protein conjugate was also evaluated in both gram-negative and gram-positive bacteria. Bovine serum albumin (BSA) protein validated our results obtained with BLA. To mimic the physiological condition, we also demonstrated the interaction of AgNP with human blood serum solution that contains number of serum proteins for the evaluation of antibacterial activity of AgNP and AgNP- blood serum proteins (BSP) conjugate. Further, we also attempted to develop a suitable capping agent of AgNP to biocompatibility and achieve higher bactericidal effect with minimum interaction with proteins.

2. Materials and methods

2.1. Materials

Pure and analytical grade chemicals and reagents were used in all experiments. $AgNO_3$, NaOH, starch, and polyethylene glycol (PEG-400), Luria agar, Luria broth and Proteinase-K were purchased from Himedia, Mumbai, India. Bovine α-lactalbumin (BLA) (MW 14.2 kDa) and BSA (MW 66.4 kDa), Sodium cacodylate, 1-Anilinonaphthalene-8-Sulfonic Acid (ANS) were procured from Sigma, India. Triton-X was purchased from Merck, India. Milli-Q water was used in all the experiments. The bacterial strains *Escherichia coli* (MTCC No. 1687), *P. aeruginosa* (MTCC No. 1688) and *Bacillus subtilis* (MTCC No. 441) were purchased from IMTECH, Chandigarh, India. All the glassware and plastic ware were purchased from Borosil (India) and Tarson (India), respectively.

2.1.1. Synthesis of AgNPs

We synthesized AgNP of desired size using 0.5 mM $AgNO_3$, 0.5% (w/v) starch and 5 mM of NaOH in 20 ml solution. Starch was dissolved in Milli-Q water by heating in microwave oven. After cooling to room temperature, $AgNO_3$ was added. This solution was heated at 90 °C with continuous stirring for 5 min and NaOH was added. The solution temperature was further raised to 100 °C and reaction was continued for 30 min. The conversion of transparent solution into yellowish brown color indicates formation of silver oxide while clear yellow color indicates conversion of silver oxide to silver nanoparticle (AgNP). For synthesis of PEG capped AgNP of equivalent size, starch was replaced with PEG (0.5% w/v) as capping agent.

2.1.2. Characterization of AgNPs

The surface plasmon resonance of AgNP was monitored by measuring the UV–vis spectrum of NPs. The NPs solution was diluted five folds with Millipore water. UV–vis spectral analysis was recorded by a UV–vis spectrophotometer Lambda-35 (Perkin Elmer) in a range of 200–700 nm, using quartz cuvette of a path length of 1 cm. Hydrodynamic size and zeta potential of AgNP was analyzed by Dynamic Light Scattering analyzer (Malvern nano-ZS) using the method previously described by Murdock et al., 2008 [15]. Field emission scanning electron microscopy (FESEM) (Nova NanoSEM/FEI) was used for analyzing surface morphology at WD 5.5 mm, voltage 10 kV and at 100,000X magnification. TEM (Jeol) was used to analyze the size and shape of AgNP sample. The X-ray diffraction pattern of AgNP powder was (XRD) analyzed by an X-ray diffractormeter (Rigaku ULTIMA IV). in the range of 25–90° with scanning speed of 2θ/min. The data was Gaussian fitted and matched with silver sample of JCPDS card No 04-0783.

2.1.3. UV/vis spectroscopic analysis

The $AgNP_{ST}$ (10 μM) was mixed with 10, 20, 50 μM of BLA in the sodium phosphate buffer (pH 7.4, 20 mM). The sample was incubated for 2 h at 25 °C and spectrophotometrically measured in the range of 200–700 nm. Baseline correction was done with respect to buffer.

2.1.4. Dynamic light scattering analysis

To obtain the hydrodynamic size distribution of $AgNP_{ST}$-BLA conjugate, the interaction was performed using 10 μM AgNP and different concentration of BLA (10,20,40,60 and 80 μM). All the samples were filtered using 0.22 μm membrane filter to remove any dust and aggregate in suspension. The samples were incubated at 25 °C and analyzed using a Malvern zeta sizer (ZS-nano). The data were represented in the terms of Volume percent with respect to size.

2.1.5. Zeta potential analysis

To analyze the stability of $AgNP_{ST}$, BLA, and $AgNP_{ST}$-BLA, the samples were analyzed by a Malvern zeta sizer (ZS-Nano). The colloidal suspension of $AgNP_{ST}$, BLA, $AgNP_{ST}$-BLA conjugate were incubated for 2 h and ζ-potential was calculated by electrophoretic mobility of particle using DTS 7.0 software (Smoluchowski equation). The data obtained by analyzing the interaction of BLA with AgNP was plotted with respect to BLA concentration. The BLA deposition on the nanoparticle surface with time due to interaction was analyzed by measuring zeta potential up to 35 min and plotted with respect to time.

2.2. Fluorescence spectroscopy

To analyze the conformational change in BLA due to interaction with $AgNP_{ST}$ steady state tryptophan (Trp) fluorescence intensity of BLA was recorded by using Cary eclipse fluorescence spectrometer (Agilent Pvt. Ltd.). Intrinsic fluorescence intensity of BLA recorded in the range of 300–400 nm by exciting the sample at 280 nm and using emission slit width 10 nm. The appropriate base line correction of test sample was performed

2.2.1. Surface hydrophobicity analysis

The surface hydrophobicity of protein was analyzed by measuring protein-bound 1-anilinonaphthalene-8-sulfonic acid (ANS) fluorescence. ANS is a well-known extrinsic fluorophore which binds with the non-polar residues/patches of the protein surface and fluoresces intensely. For surface hydrophobicity analysis, $AgNP_{ST}$-BLA conjugate (protein concentration of 10 μM) was incubated with 200 μM of ANS for 20 min. The samples were excited at 350 nm and protein-bound ANS fluorescence was recorded between 400 and 600 nm. The excitation and emission slit width of 5 and 10 nm was used, respectively.

2.2.2. Circular dichrosim measurement

The secondary structure of BLA was analyzed by measuring circular dichroism (CD) spectroscopy (Jasco 815) under constant N_2