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Bovine serum albumin interacts with silver nanoparticles with a "side-on" or "end on" conformation

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ABSTRACT

As the nanoparticles (NPs) enter into the biological interface, they have to encounter immediate and first exposure to many proteins of different concentrations. The physicochemical interaction of NPs and proteins is greatly influenced not only by the number and type of proteins; but also the surface chemistry of NPs. To analyze the effects of NPs on proteins, the interaction between bovine serum albumin (BSA) and silver nanoparticles (AgNPs) at different concentrations were investigated. The interaction, BSA conformations, kinetics and adsorption were analyzed by UV–Visible spectrophotometer, dynamic light scattering (DLS), FT-IR spectroscopy and fluorescence quenching. DLS, FTIR and UV–visible spectrophotometric analysis confirms the interaction with minor alterations in size of the protein. Fluorescence quenching analysis confirms the side-on or end-on interaction of 1.5 molecules of BSA to AgNP. Further, pseudo-second order kinetics was determined with equilibrium contact-time of 30 min. The data of the present study determines the detailed evaluation of BSA adsorption on AgNP along with mechanism, kinetics and isotherm of the adsorption.

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1. Introduction

The interaction of nanoparticles (NPs) with protein corona is necessary to understand for their biomedical applications [1,2]. After the administration of NPs into the biological system, they are getting exposed to several biointerfaces – mainly proteins of different size and quantities. Ultimately, it is NP-protein complex – not NPs alone – which decides the final biological responses [3]. Exploring the effects of interaction (activity, conformation and stability of the interacting proteins; surface property of NPs) and subsequent nature of interaction (affinity, interaction forces and binding sites) are the topics of high relevance for designing the nanomaterials for the future applications in biological system [4].

The tight and specific or nonspecific binding of proteins with nanomaterials forms a surface coating termed as protein corona [5,6]. This protein corona formation acts as the biological identity of NPs to determine their functionality and reactivity [7,8]. The adsorptivity and composition of corona on nanomaterials is dependent on time, size, shape, surface properties, chemical composition, surface charge, surface hydrophobicity, etc. – of nanomaterials [9–12]. Although, several studies have investigated the effects of surface properties on protein and other bio-interface adsorption, most of them have used protein as a coating material for stable nanomaterials with more biological acceptance [4,13,14]. Little is known about the site-specific binding, number of adsorbed proteins, adsorptivity percentage, equilibrium time, contact-time analysis, kinetics and isotherm analysis for NPs-protein interactions.

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Recently, nanotechnology has attracted more focus in the field of biotechnology; primarily silver nanoparticles (AgNPs) have attracted considerable interest in the field of food, agriculture and medicine [15,16]. Because of the rapid increase in the usage of silver nanoparticle in different sectors e.g. food, drug, agriculture, water quality management etc., it is likely that AgNP will interact with proteins; hence it is imperative to understand its fate for AgNP-protein interaction.

In our earlier study, we have synthesized AgNPs by thermal co-reduction approach and have analyzed its microbial and cellular toxicity in different microbial (*Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*) strains by evaluating the zone of inhibition, minimum inhibitory concentration, minimum bactericidal concentration, growth and death kinetics [17]. Additionally, its cytotoxicity was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in two different cell lines – lung cancer and colon cancer cell lines – which gave an IC_{50} value of 7.1 ppm and 5.3 ppm respectively. The AgNPs synthesized by thermal co-reduction approach have shown significantly more toxic effects for microbes as well as cell lines. So it is important to understand the fate of AgNP – synthesized by thermal co-reduction approach – for protein interaction. Authors have designed this experiment further to analyze the BSA interaction studies for smaller nano-silver – which was found to be more toxic for cells. It can be noted that, other than physical approach the nanomaterials-protein interaction can be better studied by docking or other computational approaches – as we have performed earlier for nano-titania [18] – but unavailability of protein data bank (pdb) file is limiting factor for *in silico* analysis.

In the present work, a combination of UV–Visible spectrophotometry, dynamic light scattering (DLS), Fourier transform infrared (FT-IR) spectroscopy, fluorescence quenching, kinetics and isotherm analysis techniques were applied to characterize the AgNP interaction with bovine serum albumin (BSA). It can be noted that, authors have chosen BSA because structurally it resembles with human serum albumin (HSA) which is found in human – so, BSA interaction studies could give us an insight into interaction of nano-silver with HSA. Also, it can be easily extracted with 99% purity and its conformation is not affected with slight change in temperature or pH. UV–Vis spectroscopy and FT-IR were used to study change in BSA characteristic peaks before and after interaction. DLS was used to monitor the increase in protein size due to its adsorption on AgNP. FT-IR also characterized the binding of AgNP at α-helix or β-sheet of BSA protein. Fluorescence quenching further determined the possible binding sites by quantifying the quenching of fluorescence from certain chromophores in the BSA structure by AgNP. Circular Dichroism (CD) spectroscopy characterized the secondary structure of the adsorbed BSA. Additionally, UV–Vis spectrophotometry was used to analyze contact-time equilibrium, kinetics and isotherms of BSA-AgNP interaction or adsorption of BSA on AgNPs. Results from individual techniques were collectively examined to understand the mechanisms of interaction.

2. Experimental section

2.1. Chemicals

All reagents used were of analytical grade and were used without further purification. Throughout the procedures, double deionized (DI) water (with a measured resistivity of 18.2 MΩ cm^{-1}) was used.

2.2. Silver nanoparticle synthesis and characterization

Silver nanoparticles were synthesized by our previously standardized protocol i.e. thermal co-reduction approach [17]. Silver nanoparticles of size 60 nm were chosen for further analysis.

2.3. Characterization

The nanoparticle suspensions were characterized by following method as given below.

2.4. Transmission electron microscopy

Samples for transmission electron microscopy (TEM) analysis were prepared by drop-coating AgNPs suspension (8 lg/ml) on carbon-coated copper grids. The suspension on the TEM grids was allowed to dry prior to measurement. TEM measurements were performed at an accelerating voltage of 120 kV (Model 1200EX, JEOL Ltd., Tokyo, Japan).

2.5. Dynamic light scattering

The average hydrodynamic size, size distribution and zeta potential of AgNPs in suspension were determined by dynamic light scattering (DLS) and phase analysis light scattering respectively using a Zetasizer Nano-ZS equipped with 4.0 mW, 633 nm laser (Model ZEN3600, Malvern Instruments Ltd., Malvern, UK).

2.6. BSA solution preparation

BSA was procured from Sigma-Aldrich, Bangalore, India with 99% purity and was used without any further purification. The stock solution of the protein was prepared in 10 mM sodium phosphate buffer solution of pH 7.4 and stored at 4 °C. All the samples were prepared in 10 mM phosphate buffer solution at pH 7.4.

2.7. BSA with AgNP suspension

AgNP (0.5, 1, 1.5, 2, 2.5 μM) was suspended in phosphate buffer and then sonicated to obtain a homogeneous suspension. BSA of concentration (10 μM) in phosphate buffer was used. A mixture of ratio 1:1 was mixed and incubated for 60 min. This solution was further subjected to different instrumentation techniques to analyze interactions.

2.8. Absorption spectra

The absorption spectra of different AgNPs-BSA suspension were recorded from 200 to 800 nm with an interval of 2 nm using a 5 nm slit-width (AU2701, Systronics Inc., India). Absorption blank was taken as phosphate buffer, and was subtracted from the absorption spectra of the relevant samples to correct the influence of the background [19].

2.9. Dynamic light scattering

The particle size distribution of AgNPs, BSA protein, AgNP-BSA interacted complex were performed by dynamic light scattering (DLS, ZetaSizer; HORIBA Instruments Pvt Ltd., Singapore) by making a clear and uniform suspension as described [20]. The scattering angle was kept at 173° with holder temperature of 25.2 °C, monodispersion medium viscosity of 1.0 mPa s, and count rate of 1247 kcps. The analyzer used was HORIBA SZ-100 for windows [Z Type] version 2.0. It can be noted that, the AgNP stock suspension (2.5 μM) was added to the protein stock solution (10 μM) in the ratio of 1:1. The measurements were taken in triplicates.

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