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Protein nanoparticle interaction: A spectrophotometric approach for adsorption kinetics and binding studies

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ABSTRACT

Investigating the protein nanoparticle interaction is crucial to understand how to control the biological interactions of nanoparticles. In this work, Model protein Bovine serum albumin (BSA) was used to evaluate the process of protein adsorption to the gold nanoparticles (GNPs) surface. The binding of a model protein (BSA) to GNPs was investigated through fluorescence quenching measurements. The strong affinities of BSA for GNPs were confirmed by the high value of binding constant (K_s) which was calculated to be 2.2×10^{11} L/mol. In this consequence, we also investigated the adsorption behavior of BSA on GNPs surface via UV–Vis spectroscopy. The effect of various operational parameters such as pH, contact time, initial BSA concentration, and temperature on adsorption of BSA was investigated using batch adsorption experiments. Kinetics of adsorption was found to follow the pseudo-second order rate equation. The suitability of Freundlich and Langmuir adsorption models to the equilibrium data was investigated. The equilibrium adsorption was well described by the Freundlich isotherm model. The maximum adsorption capacity for BSA adsorbed on GNPs was 58.71 mg/g and equilibrium constant was 0.0058 calculated by the Langmuir model at 298 K and pH = 11.0. Thermodynamic parameters showed that the adsorption of BSA onto GNPs was feasible, spontaneous, and exothermic.

1. Introduction

Gold nanoparticles (GNPs) have attracted increasing attention within recent years due to their size-tunable optical properties [1]. Different methods have been developed for precisely controlled synthesis of GNPs with specific sizes, shapes, as well as their surface chemistries can also be controlled [2]. Such development has opened a gate for various biological applications of GNPs, such as catalysis, chemical sensing, imaging, and drug delivery [1–5]. The interactions of GNPs with biological system not only require the physiochemical property control during the synthesis but also an elementary understanding of GNP–biological interactions. Introduction of GNP into a biological medium leads the formation of protein corona on GNP surfaces [6]. The surface charge of the GNPs can be significantly changed by the adsorption of proteins to the GNP surface, which guides NP fate and transport in biological systems [7]. Therefore, the biological identity of NPs largely depends on the composition of the protein corona. A considerable amount of proteins can be adsorbed and "trapped" on GNP surfaces, due to their extremely large surface area to volume ratios [6,8–12]. GNPs' coupled biomolecules have accessibility to almost every organ in vivo [13,14]. The coupled interaction can provides an insight understanding of NP–biological interaction, which is crucial for knowing GNPs' potential in biomedicine. The dynamic nature of protein corona–NP complex can be explored by certain parameters i.e. binding affinities and rates of protein adsorption/desorption. These parameters are important for understanding the cellular recognition of protein corona–NP complex [15]. Thermodynamic and kinetic study of protein nanoparticle interaction is essential to develop a framework that explores the response of cellular systems against protein corona–NP complex, depending on their size, shape, and charge.

In this work, we have investigated the binding parameters of protein-GNPs interaction along with the thermodynamics, and kinetics of adsorption of model protein, bovine serum albumin (BSA) on to the GNPs surface. Bovine serum albumin (BSA) is a soft

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globular protein, which constitute 52–62% of the total blood plasma fraction protein [16]. BSA involved in various physiological function such as maintaining the osmotic pressure and pH of blood, endogenous and exogenous transport of fatty acids, metals, amino acids, steroids and drugs [17]. The reversible binding ability of BSA molecules with substance especially with negatively charged substances ensures BSA as very good transporting agent [18]. The adsorption ability of protein on surfaces constitutes a vital research field, which is not limited to the research area such as protein purification [19,20] design of food processing equipment, biocompatibility [21], and biosensors [22]. Furthermore, from a more fundamental research viewpoint, the protein adsorption process is of concern due to the complex nature of the system. For in-depth understanding of protein adsorption process, one would like to find out how the protein concentration, pH, buffer medium, ionic strength etc. can influence the protein adsorption process. Therefore, various studies have been carried out to examine the effect of such experimental conditions on protein adsorption [23–26]. In this context, the conformational behavior of proteins in a protein–nanoparticle complex system is being a major challenge to investigate, which involves the denaturation of their tertiary and secondary structures due to protein adsorption [27]. The interaction between citrate stabilized GNPs and BSA proteins have been studied [20]. More recently, the specific interaction between GNPs and human plasma proteins have been figured out by De Paoli Lacerda et al. [28]. Moreover, GNPs can be used as a probe to investigate the conformational change of protein [29]. Evaluating the influence of the protein binding on GNPs surface provides the information of possible change in protein conformation after bioconjugation [30]. However, thermodynamic parameters such as temperature and pH can act as switch for the disruption in protein conformation that could lead to serious health implications such as cancer, diabetes, hormonal imbalance and cardiovascular diseases [31]. Consequently, these parameters can have a key influence on the nano–bio interfaces. In this regard, the spectroscopic investigation of the GNPs-BSA interfaces, as a function of pH and temperature by three different spectroscopic techniques: LSPR, fluorescence and SERS have been done by McClellan et al. [31].

In this work, we investigated the thermodynamics and kinetics of a model protein, bovine serum albumin (BSA), adsorbing to the GNPs surface. GNPs were selected as a model nanoparticle due to their interesting optical properties. Similarly, BSA is an important model protein, because it is an abundant plasma protein in mammals and highly stable. BSA contains tryptophan residues, which makes it absorb and fluoresce light at characteristic wavelengths. These characteristics are useful for studying protein binding using spectroscopic measurements. Herein, we report the effect of pH, temperature, and concentration on adsorption equilibrium and rate of adsorption of BSA on GNPs surface. Kinetic measurements were assessed at different BSA concentration and evaluated by the pseudo-first order, pseudo-second order, and intraparticle diffusion model. Similarly the equilibrium point adsorption for GNPs-BSA has been evaluated by Freundlich and Langmuir models. Thermodynamic parameters such as activation energy (E_a), the changes in free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) for the adsorption process has also been calculated.

2. Experimental methods

2.1. Reagent and chemicals

Hydrogen tetrachloro aurate (III) ($HAuCl_4.3H_2O$, 99%), trisodium citrate dihydarte ($HOC(COONa)(CH_2COONa)_2.2H_2O$), bovine serum albumin lyophylised powder (96%) were procured from Sigma Aldrich. Sodium borohydride and all other chemicals used were of analytical grade. Water was purified with a Millipore Mili system.

2.2. Synthesis of gold nanoparticles GNPs and interaction with BSA

Gold nanoparticles (GNPs) were synthesized using modified turkevich method [32]. For a typical preparation of GNPs, 0.25 mM of $HAuCl_4.3H_2O$ dissolved in 25.0 ml of millipore water and 0.25 mM trisodium citrate added with continuous stirring for making a homogenous solution. A freshly prepared 0.1 M aqueous sodium borohydride added dropwise to acquire the desired concentration. In our experiment, we maintained the concentration of $NaBH_4$ 1.0 mM in the aqueous solution. The solution turned dark ruby red immediately, which confirmed the formation of GNPs. As prepared GNPs were centrifuged at 1000 rpm for 20 min. The interaction of BSA with GNPs have been performed by dissolving the purified GNPs in Millipore water followed by the addition of different concentrations of model protein BSA (100 mg/L–500 mg/L) at various experimental pH (7.4–11.0). As prepared BSA-GNPs conjugates incubated overnight at 4 °C to ensure maximum conjugation and thereafter analyzed with UV–Visible spectrophotometer.

2.3. Steady-state fluorescence quenching measurements

For the fluorescence quenching measurements, the emission of BSA was measured at a constant concentration (500 mg/L) in the presence of an increasing concentration of gold nanoparticles (0–10 nM). The GNPs–BSA solutions were incubated overnight at 4 °C to ensure equilibrium in 5.0 ml glass vials. Before the fluorescence of BSA was measured, solutions were allowed to stand at room temperature for 30 min. The samples were then transferred to a quartz cuvette, and their fluorescence spectra were acquired in the range of 300–460 nm when excited at 280 nm. Because the fluorescence of BSA protein adsorbed to the surface of the GNPs is quenched, and the observed fluorescence is due to the free BSA in the solution.

2.4. Adsorption studies

Adsorption studies were performed, using batch technique, by equilibrating 1 mg/L of GNPs with V/m = 1 L/g with five different concentrations of 100–500 mg/L of BSA. The experiments were also conducted at three different reaction temperatures (298, 308 and 318 ± 1 K), and four different pH (7.4, 8.4, 9.4, 11.0 ± 0.1) using an initial protein concentration of 500 mg/L. For these investigations GNPs solution (1 mg/L) was contacted with 5.0 ml of solution containing known concentration of BSA and the solution was kept stirred in a thermostatic shaker adjusted at the desired temperature as a function of the time. After interval time, the shaker is stopped and the solution was analyzed using UV–visible spectrophotometer in order to determine the amount of BSA adsorbed (Supporting Information). The amount of protein adsorbed onto GNPs at any time, qt (mg/g) and the percentage adsorption (P) were calculated from the expressions:

$$q_t = (c_o - c_t)\frac{V}{m} \quad (1)$$

$$P = \left(\frac{(c_o - c_t)}{c_0}\right) \times 100 \quad (2)$$

where C_0 is the initial concentration, C_t is the concentration at time t (mg/L) of BSA in solution, V the volume (L) and m is the weight (g) of the adsorbent.