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Interaction of biosynthesized gold nanoparticles with BSA and CTDNA: A multi-spectroscopic approach

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ABSTRACT

The interaction between biosynthesized gold nanoparticles (GNP) with bovine serum albumin (BSA) and calf thymus DNA (CTDNA) was investigated from a multi-spectroscopic approach. The apparent binding constant (K) were 2.69×10^4 L/mol and 15.84×10^4 L/mol at 293 K, respectively for BSA and CTDNA, and the number of binding sites were ~1. According to the Van't Hoff equation, the thermodynamic parameters were calculated ($\Delta H = -119.496$ kJ/mol, $\Delta S = -320.92$ J/mol/K for BSA and $\Delta H = -225.89$ kJ/mol; $\Delta S = 673$ J/mol/K for CTDNA) and the results indicated hydrogen bonds and van der Waals forces are the main stabilizing force in both of the BSA-GNP and CTDNA-GNP complex. The average binding distance ($r = 4.45$ nm) and the critical energy transfer distance ($R_0 = 2.94$ nm) between GNP and BSA were also evaluated according to Förster's non-radiative energy transfer theory. What is more, UV–Visible, and circular dichroism spectra showed that the addition of GNP changed the secondary structure of BSA and led to a decrease in α-helix. Circular dichroism spectra also indicated conformational changes of CTDNA in the presence of the GNP. Furthermore, the GNP induces small changes in DNA viscosity and melting temperature which is indicative of groove binding mode of GNP with CTDNA.

1. Introduction

Recently research of nanomaterials has been grown up continuously, which can be used in a large number of industrial and biomedical applications [1]. Semiconductor nanomaterials have potential applications in biotechnology and life sciences as they possess novel optical, electronic, and chemical properties [2]. So, due to the immense biological importance of nanoparticles [3–9] the influence of nanoparticles on biomolecules has attracted lot attention and the study on the interaction between nanomaterials and bio-macromolecule (serum albumins or DNA) becomes a key point in interdisciplinary science. Accordingly, it has prime importance to realize the adverse influence of nanomaterials on biomolecules.

Serum albumins are the most abundant proteins in plasma [10] and they have many physiological functions [11] and play an essential role in binding, transport, and delivery of a wide range of endogenous and exogenous compounds to their target organs [3,4]. Among the serum albumins, BSA has a wide range of physiological functions involving binding, transport and delivery of fatty acids, porphyrins, bilirubin and steroids, etc. BSA contains 582 amino acid residues with a molecular weight of 69,000, and two tryptophan moieties at positions 134 and 212 as well as tyrosine and phenylalanine [12]. BSA binds non-covalently with drugs or inorganic materials for the efficient delivery of drugs to the affected area in the body. The binding properties of BSA with nanoparticles have been already investigated by many researchers and which are useful for understanding the effect of nanoparticles on the structures and functions of BSA [3–6,13–16]. BSA has been selected as a protein model in our study as it is water soluble in nature which is essential for interaction studies [17,18], unusual binding properties [19], and owing to its similarity to human serum albumin [20].

Nucleic acid is the primary intracellular target of antitumor drugs, owing to small molecules interaction with DNA and then damaging the formation of DNA in cancer cells [21]. So, the exploration of small molecules binding to DNA is of prime interest and it has also importance in rational design of powerful and selective new anticancer pharmaceuticals [22,23]. Small molecules can interact with DNA via three non-covalent modes: (i) electrostatic interaction, (ii) groove binding, and (iii) intercalation between the base pairs [24]. Among the three modes, the most effective mode is intercalative binding. Now-a-days, studying the nature

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of binding of nanoparticles to biomolecule like DNA is an active research area.

Gold nanoparticles have many unique and attractive properties, such as excellent conductivity, size-dependent properties, optical properties, non-toxicity, and their capacity for facile and highly variable functionalization. Among all nanostructured materials, GNP has attracted precise interest due to their stability, biocompatibility, surface plasmon resonance effect, and unique catalytic activities [25]. GNP has significance in the field of bio-nanotechnology as biomarkers, biosensors, and cancer diagnostic [26–29], Owing to the unique optoelectronic properties with their controlled size and morphology. GNP enhances the *in vitro* activities of antibacterial drugs [30,31] and what is more, GNP shows antimicrobial activities due to its cytotoxicity towards various bacterial cells [32].

Although there are reports on the study of interaction between various nanomaterial and biomolecules but there is no report available regarding the interaction between biosynthesized GNPs with BSA and CTDNA. In the featured work the interaction between biosynthesized GNP with BSA and CTDNA was investigated *in vitro* using different techniques UV–Vis absorption, fluorescence, and CD spectroscopy, as well as viscosity measurements and DNA melting techniques. We hope that this work can benefit further understanding of the binding mechanism of GNP with BSA and CTDNA.

2. Experimental

2.1. Materials

Tris–base, and EDTA were purchased from Merck, Germany and ethidium bromide (EB), BSA, CTDNA were purchased from Sigma Chemicals, USA. Tetra chloro auric acids and other molecular biology grade fine chemicals were purchased from SRL, India. All the other chemicals were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

Fluorescence spectra were recorded on Agilent Technologies Cary-Eclipse Fluorescence Spectrophotometer well equipped with attach Cary Temperature controller. The absorption spectra were obtained from a Cary 100 UV–VIS Spectrophotometer Agilent Technologies. Circular dichroic spectra were analyzed by a Jasco J-815 CD spectrometer, and viscosity was measured in Brookfield micro viscometer.

2.3. Methods

2.3.1. Synthesis and characterization of GNP

GNP was synthesized by using cell filtrate of a fungus, *Aspergillus foetidus* and 1 mM final concentration of tetra chloro auric acid solution. The biosynthesized GNP was characterized by using several biophysical techniques such as UV–Vis spectra, Dynamic light scattering, Zeta potential measurement, Fourier transform infrared spectra, X-ray Diffraction study, Atomic force microscopy, Scanning electron microscopy, energy dispersive X-ray spectrum, and Transmission electron microscopy as mentioned in our previous report [33]. Mycosynthesis is an eco-friendly, cost effective as well as an alternative option of chemical synthesis. In the process of synthesis reducing agent and capping agent are not required to confer stability to the nanoparticles as extracellular protein alone performs the both task.

2.3.2. Spectrophotometric measurements

The stock solution of BSA was prepared by dissolving BSA in a Tris–HCl (50 mM, pH 7.4) buffer to make the concentration as 1 μM. Spectral changes of 0.1 μM BSA was monitored after adding different concentrations of GNP (0, 20, 40, 60, 80, 100, and 120 μM) by recording the UV–Visible absorption (250–500 nm). The CTDNA solution was prepared by dispersing an appropriate amount of CTDNA in Tris–EDTA buffer (0.1 μM, pH 7.4) solution with stirring for 12 h at below 4 °C. Absorption experiments were carried out by keeping constant CTDNA concentration 50 μg/ml whereby varying the GNPs concentration (0, 20, 40, 60, 80, 100, and 120 μM). Spectral changes of CTDNA (50 μg/ml) were monitored after adding different concentrations of GNP (0–120 μM) by recording the UV–Visible absorption in the range of 200–400 nm.

2.3.3. Fluorescence measurements

The intrinsic emission of 1 μM protein was seen at the excitation wavelength of 280 nm. The experiments were repeated in the presence of different concentrations of GNP (0, 20, 40, 60, 80, 100, 120 and 140 μM). All experiments were also carried out at 293, 300, 303, and 310 K. The solution of EB was prepared by dissolving EB in deionized water. The fluorescence of EB is remarkably enhanced after intercalation of EB in between the base pairs of DNA [34]. The assay of EB displacement was performed as reported earlier [35]. At first, DNA (50 μg/ml) was added to 10 μg/ml aqueous EB solution and maximum quantum yield for EB was achieved at 270 nm, so this wavelength has been selected as the excitation radiation for samples at 293, 300, 303, and 310 K in the emission range of 550–700 nm. To the solution containing EB and DNA different concentrations (0–120 μM) of GNP was added successively.

2.3.4. Circular dichroism spectroscopy

The far-UV CD region (190–260), which corresponds to peptide bond absorption, was analyzed to give the content of the regular secondary structure in BSA. Protein solutions of 0.01 μM were used to obtain the spectra with and without incubation at different concentrations of GNP (0, 33.3, 66.6, 99.9, and 133.2 μM) for at least 2 min. Circular dichroism spectra showed changes in the structure of DNA, which were monitored in the region of 210–320 nm. Variable concentrations of GNP (0, 37.5, 75, 112.5, 150, and 187.5 μM) were added to 50 μg/ml of CTDNA at 303 K to study the CD spectra.

2.3.5. DNA melting experiments

CTDNA (50 μg/ml) was taken in a quartz cell and 30 μM of GNP was added. Since analyzed profiles were almost linear in the melting region, melting temperature (T_m) was determined as the average of starting and final temperatures of the melting process.

2.3.6. DNA viscosity measurements

The CTDNA concentration was fixed (50 μg/ml) and GNP concentration was varied (0–120 μM). 500 μl of CTDNA sample in presence (0–120 μM) and absence of nanoparticles were taken to monitor the viscosity in rheometer at 298 K. The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ where η_0 and η are the specific viscosity contributions of DNA in the absence (η_0) and in the presence of the nanoparticles (η), were plotted against $1/R$ (R = [DNA]/[complex]).

3. Result and discussion

3.1. UV–Vis spectral measurements

The UV–Vis absorption spectra of BSA in presence of increasing concentration of GNP (0, 20, 40, 60, 80, 100 and 120 μM) are measured at stimulated physiological condition. In presence of increasing concentration of GNP the absorption spectra of pure BSA