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New insight into the binding interaction of hydroxylated carbon nanotubes with bovine serum albumin

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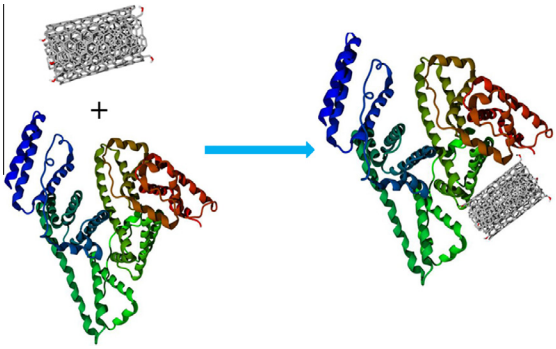
HIGHLIGHTS

- Binding interaction of HO-MWCNTs with BSA was investigated.
- The binding site of HO-MWCNTs on BSA was near to domain II and domain I of BSA.
- HO-MWCNTs acted as a pusher to increase the rate of fibrillation of BSA.
- The ligand binding and unfolding of BSA were also affected by HO-MWCNTs.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to understand the effects of carbon nanotubes on the structural stability of proteins, the ligand-binding ability, fibrillation, and chemical denaturation of bovine serum albumin in the presence of a multi-walled hydroxylated carbon nanotubes (HO-MWCNTs) was characterized by UV–vis, circular dichroism, fluorescence spectroscopy and molecule modeling methods at the molecular level. The experiment results indicated that the fluorescence intensity of BSA was decreased obviously in presence of HO-MWCNTs. The binding interaction of HO-MWCNTs with BSA led to the secondary structure changes of BSA. This interaction could not only affect the ligand-binding ability of BSA, but also change the rate of fibrillation and denaturation of BSA. This work gave us some important information about the structures and properties of protein induced by carbon nanotubes.

Introduction

At the interface of nanomaterials science and biology, the development of carbon nanotubes (CNTs) is one of the most interesting advancements [1]. Owing to their unique and outstanding properties, CNTs have many potential applications in the fields of nanobiotechnology and nanomedicine including molecular imaging, disease diagnosis, advanced drug and gene delivery, and bimolecular assembly [2–4]. Due to the great deal of applications of CNTs, the safe use of CNTs *in vivo* applications requires a clear understanding of CNTs interface. When CNTs enter the biological fluids, proteins always surround CNTs. The interactions of CNTs with proteins may change the environmental and biological activity of CNTs surface. On the contrary, the surface interactions of proteins with CNTs may be associated with some conformational changes of proteins and affect the biological function of proteins [5]. Therefore, the understanding of the fundamental interactions of CNTs with some blood proteins is of recent interest in the field of biological research [6].

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Serum albumins, the most abundant proteins of blood plasma, constitute the majority of the plasma fluid and play important roles in the transportation and disposition of several endogeneous and exogeneous compounds [7]. As the major transporter-binding proteins, serum albumins are often considered as models for studying the binding interactions of physiological substances with protein *in vitro*. In recent years, many reports have emerged about the interactions of CNTs with proteins at the molecular level [6,8,9]. Bomboi et al. have studied the interaction of a single walled carbon nanotubes with lysozyme [6]. Valenti et al. have studied the adsorption–desorption process of bovine serum albumin (BSA) on CNTs by reflectometry [8]. Li et al. have used the molecular spectral methods to investigate out the interaction of single-walled carbon nanotubes with human serum albumin [9]. The main aim of above reports is to understand the environmental and biological activity of CNTs. Although interactions between plasma proteins and CNTs at the molecular level are reported a lot, studies on the influences of CNTs on the drug-binding ability, fibrillation, and chemical denaturation of plasma proteins are extremely deficient.

The changes of the transporting drugs and other physiological substances ability of serum albumins affect metabolism, membrane penetration, half-life and other pharmacokinetic properties of drugs. Protein fibrillation is involved in many human diseases, such as Alzheimer's, Creutzfeld-Jacob disease, and dialysis-related amyloidosis [10,11]. *In vitro*, some protein denaturations have been shown to induce amyloid associated with neurodegenerative diseases. Therefore, the studies about the influence of CNTs on the above properties of serum albumin in depth are helpful to know about the potential biological risks from CNTs and to grow awareness of the nanotoxicology of CNTs [12].

In this paper, we have investigated the interactional mechanisms of one hydroxylated multi-walled carbon nanotubes (HO-MWCNTs) with BSA. The effects of HO-MWCNTs on the fibrillation and denaturation of BSA have also been studied. Then, the results of our work were expected to provide a better understanding on how exactly CNTs interact with biological molecule.

Materials and methods

Materials

Bovine serum albumin (A1933, lyophilized powder, ⩾98%), thioflavin T, 1-anilino-8-naphthalenesulfonic acid (ANS), and guanidine hydrochloride (GuHCl) were obtained from Sigma–Aldrich. HO-MWCNTs (OD, 8–15 nm, purity, >95%) were obtained from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences. The Tris, NaCl, etc. were all of analytical purity. The HCl (36%) were used to adjust pH of buffer solution. The BSA solution was prepared in pH 7.40 Tris–HCl buffer. In this paper, thioflavin T and 1-anilino-8-naphthalenesulfonic acid (ANS) were dissolved in methanol to prepare a stock solution (3.0 mM), which were stored at 0–4 °C. Ultrapure water was used throughout.

Procedure

BSA–HO-MWCNTs interaction studies

Fluorescence intensity were measured on LS–50B Spectrofluorimeter (Waltham, Massachusetts, USA) equipped with 1.0 cm quartz cells and a thermostat bath. BSA concentration was kept constant at 2 μM and the HO-MWCNTs concentration have been varied from 0 to 1.750 mg/L. In the fluorescence studies, excitation and emission slit width were set at 3.0 nm, respectively. Fluorescence spectra of all solution upon excitation at 280 nm were recorded from 300 nm to 500 nm. The synchronous fluorescence spectra were recorded at $\Delta\lambda$ = 15 nm or $\Delta\lambda$ = 60 nm. The UV–vis absorbance spectra were measured on a SPECORD S600 (Jena, Germany). The circular dichroism (CD) spectra were measured by a Chirascan spectrometer (Applied Photophyysics Ltd., Leatherhead, Surrey, UK) using a 1 mm quartz cell, the bandwidth was 1.0 nm. For the CD experiments, a 50 mM phosphate buffer of pH 7.40 was exclusively prepared in ultrapure water. The 2.0 μM BSA solution in presence and absence of HO-MWCNTs were recorded from 190 to 260 nm with three scans averaged and scan speed was set at 20 nm/min for each CD spectrum. The program CDNN was used to analyze CD spectra [13].

Computational modeling study

Computational modeling study was carried out to analysis the binding interaction of HO-MWCNTs with BSA. In the present study, the multi-wall hydroxylated carbon nanotubes were generated using Materials Studio 6.0 [14]. The diameter, length, number of walls and wall separation were set at 1.036 nm, 2.254 nm, 3, and 1.1347 Å, respectively. The crystal structure of BSA (PDB ID 3V03) was taken from RCSB Protein Data Bank [15]. HO-MWCNTs were docked to BSA using the Lamarckian Genetic Algorithm provided by Autodock 4.2.3 software obtained from the Scripps Research Institute [16]. During the modeling docking study, we used a grid box of 126–126–126 Å, which included the entire binding site of BSA and provided enough space for HO-MWCNTs translational and rotational walk. The maximum number of energy evaluations and GA population size were set to 2,500,000 and 150, respectively. Other docking parameters were default parameters. The docking data were further analyzed by using the Molegro Molecular Viewer software (Molegro-a CLC bio company, Aarhus, Denmark) [17].

Fibrillation experiment of BSA

BSA fibrillation was studied by incubating the sample solutions in absence and presence of HO-MWCNTs. The above sample solutions were prepared in thermostat bath at a constant temperature of 338 K. The complete incubation time was set 24 h. Portions of the incubated BSA solutions in absence and presence of HO-MWCNTs were removed periodically using Eppendorf pipette and were placed in 5 mL centrifugal tubes. The samples were monitored by fluorescence measurement. In order to monitor the fibrillation process of BSA, the fluorescence spectra of thioflavin T in BSA-HO-MWCNTs system were measured during the BSA fibrillation. The emission spectra of thioflavin T were detected using an excitation wavelength of λ_{ex} = 440 nm in the range from 460 nm to 600 nm.

Guanidine hydrochloride denaturation of BSA

To a fixed volume (3.0 mL) of BSA solution, BSA was previously incubated with different GuHCl concentration for 10 h in absence and presence of HO-MWCNTs at room temperature. BSA concentration was kept constant at 2 μM and the HO-MWCNTs concentrations have been set at 0 mg/L, 0.525 mg/L, and 1.750 mg/L, respectively. In the each BSA-HO-MWCNTs system, the GuHCl concentrations have been set at 0.0 M, 1.0 M, 2.0 M, 3.0 M, 4.0 M, 5.0 M, 6.0 M, 7.0 M and 8.0 M, respectively. After GuHCl denaturation, the fluorescence spectra of all solution upon excitation at 280 nm were recorded from 300 nm to 450 nm.

Results and discussion

The binding interaction of HO-MWCNTs with BSA

BSA has two Trp residues (Trp-134, Trp-213), which have the maximum fluorescence emission peak at 346 nm when the excitation wavelength is fixed at 280 nm. The fluorescence change of Trp