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Binding studies of hydroxylated Multi-Walled Carbon Nanotubes to hemoglobin, gamma globulin and transferrin

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ABSTRACT

Biocompatibility of nanoparticles depends on their binding behavior with biomolecules. Herein, we have reported the interaction of three different biological macromolecules such as hemoglobin, gamma globulin and transferrin with hydroxyl group functionalized Multi-Walled Carbon Nanotubes (OH-MWCNTs). Multiple spectroscopic methods were utilized to identify the binding cum structural changes in biomolecules upon their interaction. Hyperchromic effect observed in the UV–visible spectra, and the quenching behavior from fluorescence emission evidences the existence of bio-nanotube complex formation. Synchronous and three-dimensional fluorescence spectra of biomolecules, in correspondence with Trp and Tyr residues showed the possible disturbance towards their aromatic micro-environment. Changes observed in the FTIR and FT-Raman amide bands, and amino acid residue position of biomolecules upon interaction with CNTs showed the possible effect towards their secondary structure. Further studies with CD spectroscopy indicated the loss of alpha-helical structures quantitatively. The study remains significant in evaluating the biosafety profile of functionalized MWCNTs for their in vivo biomedical applications.

1. Introduction

Carbon nanotubes (CNTs) are allotropes of carbon. Based on the number of graphene layers rolled up, they are divided into single-walled (SWCNTs) or Multi-Walled Carbon Nanotubes (MWCNTs). Each nanotube contains millions of atoms and hence, their length ranges from tens of micrometers long and diameters range as small as 0.7 nm [1]. The unique and outstanding properties of CNTs paved their potential applications in the fields of nano-biotechnology and nanomedicine such as molecular imaging, disease diagnosis, advanced drug and gene delivery, and bimolecular assembly [2]. Still, the dispersion behavior of CNTs deserves a major disadvantage due to their hydrophobic nature [3]. Several functionalization methods were developed for improving their dispersion in solvents. Among which, the functionalization with biomolecules not only showed improved dispersion, but also reduces their toxicity profile [4]. Thus, their improved biomedical and environmental applications require the knowledge to be compiled in terms of the safer use of functionalized CNTs. Especially, a clear understanding of the biological interface of functionalized CNTs should be stressed enough in terms of their interaction with biological macromolecules.

Proteins are the biomolecules with several significant roles in human beings [5]. When an exogenous material enters the human physiological environment, they are immediately covered by the biological proteins. This adsorption phenomenon remains the basic key behind the understanding of the bionanoscience world [6]. But, a clear knowledge of the protein corona formation on the nanoparticle surface remains quiet challenging, due to the varying adsorption affinities, time to reach equilibrium and the concentration of over 3700 proteins in plasma [7]. Information obtained from nanoparticles–protein corona studies sounds to be significant in determining the final sub-cellular location of specific nanoparticles upon interaction with a cell and the associated diseases [8].

Similar to other nanoparticles, the biocompatibility and toxicity of CNTs also require the fundamental understandings of the conformational behaviors of the biomolecules. Because, such interactions would not only alter the environmental and biological activity of CNT surface, but also induce conformational changes in biomolecules and affect their biological function [9]. When CNTs penetrate the plasma membrane, their interactions with the oxygen transporter proteins would affect the human health. Hemoglobin plays as an oxygen carrier in red blood cells and muscle cells [10]. They help in the transport of oxygen, aid the dispersion of hydrogen peroxide, and helps in the electron transfer in human body parts and organs [11]. Gamma-globulin is a protein

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fraction of blood serum [12] and their concentration remains about 8.3–23.0 g/l. They play an important role in immune defense mechanisms. Gamma globulin contains five species of immunoglobulin (Ig): IgG, IgA, IgM, IgD, and IgE, and among them the main component is IgG (75%) produced by B lymphocytes to identify and neutralize foreign objects such as bacteria and viruses. Transferrin concentration in plasma from healthy subjects is 2.2–4.0 g/l. The fundamental role of transferrin is to control the levels of free iron in body fluids by binding, sequestering, and transporting Fe^{3+} ions [13]. In addition, transferrin can also bind to many other metals, including gallium and cobalt and hence occupies significant physiological function in terms of the iron transport in the circulatory system [14,15]. These proteins may accumulate in blood plasma and function as a diagnostic marker for a certain disease. These blood plasma proteins exhibit a wide variety of functions and have different structural properties [16].

Hence, in this paper, we have investigated the interaction of hydroxylated Multi-Walled Carbon Nanotubes (HO-MWCNTs) with abovementioned biological macromolecules. Multiple spectroscopic techniques such as UV–visible and fluorescence spectroscopy were used for studying the binding mechanism, FTIR and FT-Raman for evidencing changes in the amide position and CD spectroscopy to quantitatively investigate the loss of alpha-helical contents.

2. Materials and Methods

2.1. Chemicals

Multi-Walled Carbon Nanotubes Type 15 (–OH functionalized) with an outer diameter ranging from 30–50 nm, Batch number: T 8371597 were procured from Sisco Research Laboratories private limited, Mumbai. Hemoglobin, gamma globulins and transferrin from human were obtained from Sigma-Aldrich, USA. All other chemicals used are of higher analytical grade. Biomolecules (0.01%) were prepared with phosphate buffer of pH 7.2 (0.1 M). CNTs were dispersed using ultra-sonicator bath for 30 min in the same buffer prior to the experiment.

2.2. Surface Characterization of CNTs

Raman spectra of OH group functionalized MWCNTs were measured using a FT-Raman spectrophotometer (Bruker RFS $_{27}$, Multi RAM) stand alone model. The laser source used was Nd: YAG 1064 nm that is operated at a resolution of 2 cm^{-1}. The spectral range was reported from 4000–50 cm^{-1}. The FTIR Spectrometer (Perkin Elmer Spectrum-1), operating at a resolution up to 1.0 cm^{-1} was used to obtain FTIR spectra. The spectral range was reported from 4000–450 cm^{-1}. Structural morphology of OH-MWCNTs was studied with Scanning Electron Microscopy (FESEM-Supra55) Carl Zeiss, Germany. In addition, Energy Dispersive X-Ray spectroscopy was used to measure the metal impurities found on the surface of carbon nanotubes.

2.3. Fluorescence Quenching Studies

Fluorescence emission spectra of the blood proteins were measured by using a Spectrophotometer F-7000 FL (Hitachi, Japan). Interaction was carried out by mixing known and increasing concentrations of CNTs (2–10 mg/l) with constant biomolecules (0.01%) under orbital shaking at 150 rpm for 5 min at room temperature. Excitation wavelength was set as 280 nm. Scanning speed was maintained at 2400 nm/min. Excitation and emission slit were set as 5 nm. Emission spectrum was reported from 300 to 400 nm. Stern–Volmer (I_0/I vs. Q the concentration of MWCNTs) and Double Logarithmic plots [Log (I_0 − I) / I vs. Log Q] were done to evaluate the binding parameters.

2.4. Synchronous Fluorescence Spectra

Synchronous fluorescence spectra of the blood proteins were measured by using the Spectrophotometer F-7000 FL (Hitachi, Japan). At room temperature, the synchronous fluorescence spectra of hemoglobin, gammaglobulin and transferrin with the existence of mounting concentrations of OH-MWCNTs were investigated. In general Δλ represents the difference between the emission and excitation wavelength of biomolecules. Δλ values of 15 and 60 nm were fixed. Scan and data mode was optioned as synchronous and fluorescence. Emission wavelength for Δλ = 15 nm and 60 nm was set as 295 and 340 nm. Excitation start and end wavelength was given as 200 to 600 nm. Scan speed delay remained at 1200 nm/min and 0.0 s. Excitation and emission slit was set as 5 nm and PMT voltage as 400 V. Integration method was set as rectangular. Sensitivity and threshold remained as 1.000. The data obtained was reported from 200 to 600 nm respectively.

2.5. Three Dimensional Spectra

Three-dimensional fluorescence spectra of biomolecules were measured with the Spectrophotometer F-7000 FL (Hitachi, Japan) and the instrument parameters were maintained as follows. Measurement type remains as 3D scan, and data mode was set as fluorescence. Excitation and emission's start and end wavelength were set as 200 and 900 nm respectively. Excitation and emission sampling interval was set as 40 and 10 nm. Scan speed remains as 60,000 nm/min. Excitation and emission slit was set as 5 nm, and the PMT voltage remained as 400 V. Both, the contour and the bird's eye view plot of biomolecules in the absence and presence of OH-MWCNTs were represented. Stoke shift (Δλ nm) between the emission and excitation wavelength was calculated. The alteration in the fluorescence intensity along with a notable shift in the fluorescence emission range was elucidated.

2.6. UV–visible Spectra

The absorbance of biomolecules in the absence and the existence of increasing concentrations of OH-MWCNTs was measured with a Double Beam spectrometer (Systronics, India), at a resolution of 0.1 nm. Samples were allowed to interact with 2–10 mg/l of OH-MWCNTs in the orbital shaker at 150 rpm at 25 ± 3 °C for 5 min. Data obtained was reported in the spectral range from 200 to 600 nm for hemoglobin and from 200 to 350 nm for gammaglobulin and transferrin. Adsorption kinetics was measured by incubating 0.1 mg of biomolecules with 100 ppm of CNTs for different time intervals. The amount of biomolecules adsorbed at different time intervals 'q_t' (mg/g) on OH-MWCNTs was calculated from the following equation:

$$q_t = (C_0 - C_e)V/W$$

where 'C_0' and 'C_e' (mg/ml) are the concentrations of blood proteins at initial and different time intervals, respectively, 'V' is the volume of the reaction suspension (ml) and 'W' is the mass of OH-MWCNTs (g). All the tests were carried out in triplicate, and mean values of the results were reported.

2.7. FTIR Spectra

Fourier Transform Infrared (FTIR) spectra of the control and OH-MWCNTs interacted biomolecules were derived with a Perkin Elmer Spectrum One Spectrometer with a resolution of 1. The instrument contains Kbr and Mylar beam splitter, and the lyophilized samples were mixed with Kbr and pressed into pellets at low pressure (1.5 Psi). The spectra were measured from 4500 to 400 cm^{-1} at 25 ± 3 °C temperatures.