Journal of Hazardous Materials 292 (2015) 98–107

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Exploring the diameter and surface dependent conformational changes in carbon nanotube-protein corona and the related cytotoxicity

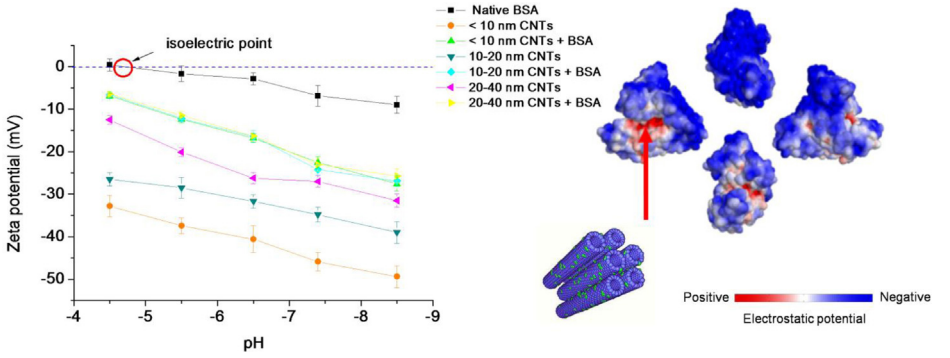
Xingchen Zhao [a], Dawei Lu [a], Fang Hao [a], Rutao Liu [b,*]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China
[b] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, China–America CRC for Environment & Health, Jinan 250100, PR China

HIGHLIGHTS

- CNT diameter and surface area govern the stability of adsorbed proteins.
- More BSA was loaded and destabilized on smaller CNTs.
- Protein corona reduces the cytotoxicity of CNTs

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form 3 February 2015
Accepted 11 March 2015
Available online 14 March 2015

Keywords:
Carbon nanotubes
Diameter
Serum albumin
Nanotoxicology

ABSTRACT

In this work, we investigated and compared carbon nanotubes (CNTs) of different diameters regarding their interaction with bovine serum albumin (BSA) and their ability to alter protein structure. BSA was exposed to CNT solutions, and the effects were assessed by utilizing fluorescence spectroscopy, UV–vis absorption spectroscopy, circular dichroism (CD) spectroscopy, transmission electron microscopy (TEM), bichinchoninic acid (BCA) and zeta-potential measurement assays. We demonstrate that CNT diameter and surface area play key roles in influencing the stability of adsorbed proteins. Results showed that the secondary and tertiary structural stability of BSA decreased upon adsorption onto CNTs, with greater decrease on smaller-diametered nanotubes. Besides, more protein was loaded onto CNTs with small diameter, reducing the cytotoxicity. This study, therefore, provides fundamental information for the influence of CNT diameter and surface on protein behavior, which may be helpful to understand toxic effects of CNTs and prove beneficial for developing novel biomedical devices and safe use of nanomaterials.

1. Introduction

The discovery of numerous carbon nanomaterials has created a new field of vision to the rapid development of

* Corresponding author. Tel.: +86 531 88364868; fax: +86 531 88364868.
E-mail address: rutaoliu@sdu.edu.cn (R. Liu).

http://dx.doi.org/10.1016/j.jhazmat.2015.03.023
0304-3894/

nanotechnology especially after the discovery of carbon nanotubes (CNTs) by Iijima [1]. Keen interest due to their unique electrical and mechanical properties is now leading to mass production and commercial application of nanomaterials, for example, nanotechnology, nanoelectronics, and composite materials [2,3]. Researchers showed that bioelectrochemical reactions can be driven by attaching proteins onto the surface of CNTs and that well-controlled aligned CNTs can be applied as immobilization matrices and as mediators for the development of biosensor devices [4]. Not just this, researchers also focus on how to form stable colloidal dispersions in various solvents including physiological solutions, in which solubility of CNTs is extremely small. Such soluble CNTs were expected to be promising as the basis for potential biotechnological and medical applications, in particular, utilized for DNA, proteins, and drug delivery [5].

The wide production, use and disposal of CNTs will inevitably lead to their exposure to human beings. Recent years, uncertainty over the potential detrimental effects of CNTs on human health and the environment has caused wide attention, especially the editorial articles on bio-effects, environmental and physical influences of nanoscaled particles was published on Science [6] and Nature [7].

Proteins are chief actors and fundamental components of all living cells. By studying the structures of proteins, we are better able to understand how they function normally and how some proteins with abnormal shapes can cause disease. Thus to investigate the interaction of protein and CNTs would be of great value and be potential to reveal the mechanism of CNT toxicity. However, data for CNT toxicity at a protein level are still scarce so far. Zhang et al. found that functionalized CNTs specifically bind to α-chymotrypsin's catalytic site and regulate its enzymatic function [8]. Mu presented the conjugation of four proteins onto diverse multi-walled carbon nanotubes (MWCNTs). The three-dimentional structure of BSA changed followed by exposure of hydrophobic groups [9]. In our previous study we showed that MWCNTs could interact with BSA through mainly electrostatic forces and could cause secondary and tertiary structure alteration of the protein [10].

Here, we used MWCNTs with diverse diameters which were shortened, and functionalized by strong-oxidative solution. We emphasized the diameter-dependent effect of CNTs with BSA, which is the major component of blood plasma and serves as a carrier of chemical substances. Fluorescence spectroscopy was used to detect the microenvironment alteration of BSA's fluorescent residues caused by CNTs. and ultraviolet–visible absorption spectroscopy was utilized to analyze protein's conformation and backbone changes. Furthermore, secondary and tertiary structural transformations were monitored by CD spectroscopy. We demonstrate quantitatively that the heart-shaped protein undergoes different degrees of unfolding as a function of CNT diameter. This work provides new insights into the impact of CNT diameter and surface area upon the secondary and tertiary structural stability of adsorbed protein as well as concerning cytotoxicity.

2. Experimental

2.1. Reagents and apparatus

MWCNTs (<10 nm, 10–20 nm and 20–40 nm) were bought from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). All MWCNTs were synthesized by chemical vapor deposition. The purity was higher than 90%, and the catalyst residue was less than 0.2%. NaH_2PO_4, Na_2HPO_4, HNO_3, H_2SO_4 and NaOH were all purchased from Tianjin Tianda Chemical Reagent Co., Ltd. (Tianjin, China). BSA was bought from Sigma Chemical Co. (St. Louis, M.O.) without further purification, and was dissolved in ultrapure water to form a 1.0×10^{-5} mol/L solution, then preserved at 0–4 °C for later use. All solutions were prepared with ultrapure water (18.25 MΩ) and adjusted the pH to 7.4 using 0.2 M phosphate buffered saline (PBS).

In this work, 8400 S Fourier transform infrared (FTIR) spectroscopy (Shimadzu, Japan) and Q600SDT thermogravimetric analyzer (TA Instruments, U.S.) were used for characterization of CNTs. The ultraviolet/visible absorbance spectra (UV–vis) were recorded on a Shimadzu UV-2450 spectrometer (Shimadzu, Japan) in a 1 cm quartz cell with reference to proper solutions. Transmission electron microscopy (TEM) images were captured using a JEM-2100 transmission electron microscope (JEOL Inc., Japan). Size of MWCNTs was characterized by a BI-200SM Dynamic/static Laser scattering system (Brooken Haven, USA.). Steady-state fluorescence spectra were taken using an FL-4600 fluorescence spectrophotometer (Hitachi Co., Ltd., Japan). The emission spectra were monitored in the range of 290–410 nm with the fixed slit width of 5 nm. Inner filter effect which is caused by the absorption of both excitation and emission radiation of the sample was taken into account in this system. CD spectra were measured by a J-810 Spectropolarimeter (Jasco, Japan) under constant nitrogen flush. The far-UV region was scanned between 200 and 240 nm with an average of two scans and also a bandwidth of 5 nm. BET surface area measurements were performed with a Quadrasorb SI unit (Quantachrome, USA) using nitrogen adsorption at 353 K. PBS buffer were used to adjust the pH to the desired value. The ζ potential was measured using a Malvern ZetaSizer NanoSeries (Malvern Instruments, UK) at 298 K.

2.2. Preparation of soluble CNTs with diverse diameters

To remove the impurities, a pristine sample of nanotubes (0.8 g) was first immersed in 1.2 L of 2 M nitric acid and stirred for 24 h. The solids were then washed twice with 2 L ultrapure water. The purified tubes were then being suspended in 200 mL of a 3:1 mixture of concentrated H_2SO_4/HNO_3 (v:v) and stirred at reflux (60–70 °C) for 24 h. The resultant suspension was poured in 800 mL of water afterwards. After being filtered and washed with 10 mM NaOH solution on a 220-nm pore filter membrane, larger MWCNTs were dried in vacuum to obtain solvable powder. We suspended the as-prepared MWCNTs at a density of 0.15 mg/mL in water then diluted into different concentrations followed by absorbance measurements at 907 nm. Absorbance vs concentration working curve was plotted as demonstrated in our previous work [10]. MWCNT solution of a certain concentration was centrifuged and the supernatant was obtained. The concentration of the surfactant with well dispersed MWCNTs was determined according to the absorbance of the aqueous solution.

2.3. Protein adsorption onto CNTs

For protein adsorption experiments, CNT stock solutions (0.1 g/L) were shaken and subjected to sonication for 5 min. CNTs were then exposed to 0.065 g/L BSA solution with 20 mM phosphate buffer to reach a final concentration of 10 mg/L in 10 mL Corning centrifuge tubes. The mixtures were then shaken on a platform shaker for 1 h at 150 rpm and 25 °C. After incubation, the mixtures were centrifuged at 7000 rpm and the supernatant was removed for protein concentration detection. BCA reagent box was bought from Biocolor BioScience & Technology Company (Shanghai, China) and was used for determination of protein solution concentration.

2.4. Cell culture and cell viability test by MTT assay

Primary astrocytes line was obtained by the method from Frangakis [11]. The cells were maintained in DMEMF12 medium (Hyclone, USA) supplemented with fetal bovine serum (10%,