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Research article

Effects of serum albumin on the degradation and cytotoxicity of single-walled carbon nanotubes

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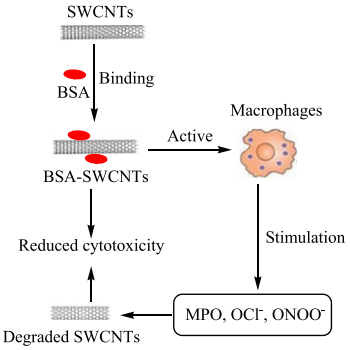
HIGHLIGHTS

- Binding of BSA could impair MPO-induced SWCNTs degradation in vitro.
- The degradation degree was more significant for BSA-SWCNTs in activated macrophages.
- Binding of BSA to SWCNTs reduced cytotoxicity.
- Biodegraded nanotubes induced less cytotoxicity than the non-degraded nanotubes.

GRAPHICAL ABSTRACT



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ABSTRACT

Neutrophil myeloperoxidase (MPO) and peroxynitrite ($ONOO^-$) can oxidatively biodegrade carboxylated single-walled carbon nanotubes (SWCNTs). The protein–SWCNTs interactions will play an important role in the degradation and cytotoxicity of nanotubes. Here, we investigated the binding of bovine serum albumin (BSA, a common and well-characterized model blood serum protein) to SWCNTs, and found that the hydrophobic and electrostatic interactions might be crucial factors in stabilizing the binding of SWCNTs with BSA. The binding of BSA could impair SWCNTs biodegradation in vitro through the competitive adsorption to nanotube. Both SWCNTs and BSA-SWCNTs were significantly degraded in zymosan-stimulated macrophages, and the degradation degree was more for BSA-SWCNTs. The mechanism for SWCNTs degradation in activated macrophages was further investigated to demonstrate the dominant participation of MPO and $ONOO^-$-driven pathways. Moreover, binding of BSA to SWCNTs reduced cytotoxicity and degraded nanotubes induced less cytotoxicity than non-degraded nanotubes. The binding of BSA may be an important determinant for the biodegradation and cytotoxicity of SWCNTs in inflammatory cells, and therefore, provide a new route to mitigate the potential toxicity of nanotubes in future biomedical applications.

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1. Introduction

Carbon nanotubes (CNTs) have a variety of potential biomedical applications, such as cancer therapy and diagnoses, carriers for

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chemotherapeutic drugs, Raman detection and imaging, and photothermal therapy of cancers [1–3]. Meanwhile, CNTs also raised large concerns about their possible adverse effects on human health. Many in vitro and in vivo studies have indicated that CNTs can develop an inflammatory response and may be cytotoxic [1–4]. CNTs are accumulated and slowly cleared in vivo due to their chemically stable abilities. Therefore, studies on the biodegradative mechanism of CNTs are of great importance in nanomedicine [5].

Enzymatic degradation of CNTs was demonstrated in recent years using heme peroxidases such as myeloperoxidase (MPO) and horseradish peroxidase [6–9]. The carboxylated single-walled CNTs (SWCNTs), but not pristine CNTs, can be degraded by peroxidase activity of these heme proteins. The interaction of heme with hydrogen peroxide (H_2O_2) leads to the formation of peroxidase reactive intermediates, which can effectively oxidize CNTs [6,7]. The degradation of CNTs into shorter CNTs can decrease their toxicity and accelerate their clearance from the body [6], and therefore significantly promote the biocompatibility of CNTs. In addition to its role in the peroxidase cycle, MPO can uniquely catalyze oxidation of Cl^- to produce hypochlorite (OCl^-) [10,11]. Both reactive radical intermediates of MPO and OCl^- are the oxidants involved in the degradation process of SWCNTs in vitro and in vivo [6,12]. SWCNTs are also oxidatively biodegraded via peroxynitrite ($ONOO^-$)-driven pathways in activated macrophages [13], where $ONOO^-$ can be generated as a result of superoxide (O_2^-) interacting with nitric oxide synthases-derived nitric oxide (NO).

Non-covalent coating of SWCNTs with proteins will affect recognition patterns, metabolic pathways and toxicity of the nanomaterials [14–18]. It has been reported that the binding of serum proteins to CNTs can greatly alter their cellular interaction pathways and strongly reduce their cytotoxicity, and thus enhance the biocompatibility of nanotubes [19–21]. Although the interactions between proteins and CNTs are believed to play an important role in the biological effects of CNTs [15–21], no previous efforts have been made to demonstrate the protein-SWCNTs interactions that influence the biodegradation and subsequent cytotoxicity of nanotubes.

In this study, we used bovine serum albumin (BSA) as a common and well-characterized model of serum proteins, and investigated the effects of BSA–SWCNTs interactions on the degradation and cytotoxicity of nanotubes.

2. Experimental section

2.1. Materials

Single-walled carbon nanotubes were purchased from XF NANO (China). Bovine serum albumin (BSA), 4-aminobenzoic acid hydrazide (ABAH), apocynin, taurine, uric acid, zymosan and MPO were purchased from Sigma-Aldrich. Carboxylated single-walled carbon nanotubes (SWCNTs) were prepared [6,8] and used throughout the study unless specified otherwise. SWCNTs in PBS (20 mM, pH 7.4) were incubated with BSA in 1:1 ratio (w/w) for 2 h with sonication for 2 min every 20 min.

2.2. Molecular modeling, docking of SWCNTs to BSA

The 3D structures of SWCNTs were generated using Nanotube Modeller software. SWCNTs were modified at the edge to contain carboxyl groups using Pymol visualization software. Then, SWCNTs were docked to the BSA-ray crystal structure (PDB ID: 3V03) by AutoDock software, as described previously [6,8].

2.3. Degradation of SWCNTs by MPO in vitro

SWCNTs or BSA-SWCNTs (0.1 mg/ml) were incubated with MPO in PBS containing NaCl (140 mM). H_2O_2 was added at a rate of 400 μM per 8 h [6,8]. Because of the loss of MPO activity and BSA in the incubation system, the enzyme and BSA was replenished after 12 h and the reaction mixture was maintained for 24 h.

2.4. MPO release, OCl^- and $ONOO^-$ generation in macrophages

Macrophages were incubated with either serum-opsonized zymosan (SOZ) or nanotubes. Then, macrophages were centrifuged and the obtained supernatant was used for the measurements of MPO, OCl^- [6,8], and $ONOO^-$ [17,22] according to these previous studies.

2.5. Assessment of SWCNTs degradation and cytotoxicity in macrophages

In the absence or presence of SOZ, cells were incubated with SWCNTs or BSA-SWCNTs for 24 h. Meanwhile, macrophages were pre-treated with either 4-aminobenzoic acid hydrazide (ABAH, inhibitor of MPO), apocynin (inhibitor of NADPH oxidase), Taurine (OCl^- scavenger), or Uric acid ($ONOO^-$ scavenger) for 1 h prior to the addition of SOZ and BSA-nanotubes. Then, the obtained samples were used to assessment of SWCNTs degradation by Raman spectroscopy [6,8]. Cellular viability was determined by the CCK-8 assay kits as described previously [19].

3. Results and discussion

3.1. The interactions of BSA with SWCNTs

Molecular docking studies were first performed to characterize possible BSA interaction sites on SWCNTs. The predicted best interaction site with lowest binding energy (−9.83 kcal/mol) revealed that LYS64, CYS75, ALA78, ARG81, GLU82, CYS91 and GLN94 were located in the binding pocket and predicted to stabilize the interaction between SWCNTs and BSA (Fig. 1).

Generally, various weak interactions may contribute to protein adsorption on CNTs, such as π-π stacking, hydrophobic and electrostatic interactions [19,20]. Our simulations revealed that polar residues, e.g., two cysteines (CYS75, CYS91) and GLN94, seemed to have significant contributions to the binding of proteins onto CNTs (Fig. 1B). Based on previous studies [14,19,20] and results herein, it could be demonstrated that these hydrophilic residues contacted SWCNTs via their nonpolar aliphatic chain, whereas the polar groups were pointing out to the water, further indicating that the hydrophobic interaction served as one of driving forces in the protein–CNTs binding. Also, the oxidized groups (carboxyl) on SWCNTs in the binding site were stabilized by electrostatic interaction with the positively charged residue, ARG81 on BSA (Fig. 1B). Therefore, these theoretical results herein illustrated that besides of the widespread π-π stacking interactions [19], the hydrophobic interactions between SWCNTs and polar residues (CYS, GLN) in BSA and electrostatic interaction of positively charged ARG residue with carboxyls on SWCNTs might be the crucial factors in stabilizing the binding of carboxylated SWCNTs with BSA.

In addition, we attempted to semiquantitatively analyze protein adsorption by SDS-PAGE and quantify the binding of BSA to SWCNTs. With the increase of incubation time, the band intensity of protein changed from light to dark (Fig. S1A), which indicated that protein content in the sediment became much bigger due to protein adsorption onto SWCNTs. The protein-binding capacity of SWCNTs at 60 min was 0.43 ± 0.02 mg BSA per mg of SWCNTs (Fig. S1B), much higher than the MPO-binding ability of SWCNTs (0.18 ± 0.02 mg per mg of nanotubes) [9]. This higher protein-binding capacity of BSA was probably related to the smaller size of BSA than MPO [8].

3.2. Binding of BSA to SWCNTs reduced the degradation in vitro

Then, MPO and $ONOO^-$-mediated SWCNTs degradation was studied. The degradation degree could be estimated on the basis of the absorption intensity at λ = 1060 nm [6,9]. Consistent with these

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