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Binding of human IgG to single-walled carbon nanotubes accelerated myeloperoxidase-mediated degradation in activated neutrophils

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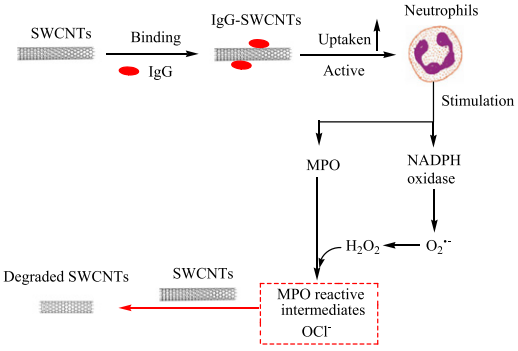
HIGHLIGHTS

- MPO was able to degrade SWCNTs.
- IgG could spontaneously bind to the surface of SWCNTs.
- The binding of IgG could impair MPO-induced SWCNTs biodegradation in vitro.
- IgG-absorbed SWCNTs stimulated neutrophils to produce MPO and OCl^-.
- The degradation degree was more significant for IgG-SWCNTs in activated neutrophils.

GRAPHICAL ABSTRACT



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ABSTRACT

The binding of protein to carboxylated single-walled carbon nanotubes (SWCNTs) was believed to play an important role in the biological effects of nanotubes. However, the effects of protein–SWCNTs interactions on the oxidative degradation of nanotubes were not stressed enough. Here, we investigated the binding of human immunoglobulin G (IgG) to SWCNTs, and found that the preferred binding site was located in the F_c region of IgG. The hydrophobic and electrostatic interactions might be the crucial factors in stabilizing the binding of SWCNTs with IgG. Through the competitive binding of IgG and myeloperoxidase (MPO) to nanotube surfaces, the binding of IgG could impair MPO-induced SWCNTs biodegradation in vitro. However, both SWCNTs and IgG-SWCNTs were significantly degraded in zymosan-stimulated neutrophils, and the degradation degree was more for IgG-SWCNTs. These results suggest that the binding of IgG may be an important determinant for MPO-mediated SWCNTs biodegradation in activated human inflammatory cells.

1. Introduction

The biotechnological and biomedical applications of carbon nanotubes (CNTs) have raised large concerns about their possible adverse effects on human health. Many in vivo and in vitro studies have indicated that CNTs can develop an inflammatory response and may be cytotoxic [1–4]. Due to their chemically inert and stable abilities, CNTs are accumulated in vivo and hardly to be cleared. Hence, studies on the biocompatibility and biodegradative mechanism of CNTs are of great importance in nanomedicine [5].

Apart from the chemical degradation of CNTs by strong acids and oxidants, enzymatic oxidative degradation of CNTs was demonstrated in recent years using heme peroxidases, such as horseradish peroxidase

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(HRP) and human myeloperoxidase (MPO) [6–9]. The carboxylated single-walled CNTs (SWCNTs) can be degraded by peroxidase activity of these heme proteins in the presence of low concentrations of hydrogen peroxide (H_2O_2). The interaction of active heme with H_2O_2 leads to the formation of peroxidase reactive intermediates, which can effectively oxidize CNTs [6,7]. The degradation of CNTs into short fragments can decrease their toxicity and accelerate their clearance from the body [6]. MPO is abundantly expressed in neutrophils that play a crucial role in the principal defense mechanisms of innate immunity [10,11]. In addition to its role in the peroxidase cycle, MPO possesses the chlorinating ability to catalyze oxidation of Cl^- to produce hypochlorite (OCl^-) [10]. 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].

The non-covalent coating of SWCNTs with proteins in vivo will inevitably affect recognition patterns, metabolic pathways and toxicity of the nanomaterials [13–18]. It has been reported that the binding of human serum proteins to CNTs can greatly alter their cellular interaction pathways and strongly reduces their cytotoxicity [16,17]. Interactions of CNTs with bovine serum albumin (BSA) can enhance their biocompatibility and the BSA-dispersed SWCNTs can be uptaken by various cells [18]. As the second most abundant protein in human blood plasma and extracellular fluid, immunoglobulin G (IgG) is an antibody isotype vital for immune system function [19–21]. It is responsible for the primary defense mechanism in humans when external antigenic compounds enter the body. Here, it coats the pathogen surfaces allowing their recognition and phagocytosis, activates the classical pathway of the complement system, and binds and neutralizes toxins [19]. Despite its role in the immune system and importance in medical applications, IgG was rarely used as a model protein in protein-SWCNTs interactions.

In this study, we investigated the influence of human IgG-SWCNTs interactions on the biodegradation of nanotubes. The current work demonstrates that the binding of IgG may be an important determinant for MPO-mediated SWCNTs biodegradation in activated inflammatory cells. Therefore, functionalization of SWCNTs by IgG aimed to target them to immune cell may provide a good platform to improve the biodegradation and biocompatibility of nanotubes in vivo.

2. Material and methods

2.1. Materials

Single-walled carbon nanotubes (purity > 90%, outer diameter: 1–2 nm, length: 0.5–2 μm) were purchased from XF NANO (China). IgG from human serum, zymosan, human myeloperoxidase (MPO) and sodium hypochlorite (NaOCl) were purchased from Sigma-Aldrich. Carboxylated single-walled carbon nanotubes (SWCNTs) were prepared [6,8], and used throughout the study unless specified otherwise.

2.2. Binding of IgG to SWCNTs

SWCNTs (0.2 mg/ml) in PBS (20 mM, pH 7.4) were incubated with human IgG in 1:1 ratio (w/w) for 1 h at 37 °C with sonication for 2 min every 15 min.

2.3. Degradation of SWCNTs by MPO

SWCNTs or IgG-SWCNTs (0.1 mg/ml) were incubated with MPO in PBS (20 mM) containing NaCl (140 mM). H_2O_2 was added at a rate of 400 μM per 12 h [6]. Because of the loss of MPO activity and IgG in the incubation system, the enzyme and IgG was replenished after 24 h [6,8,9], and the reaction mixture was maintained at 37 °C for 48 h. IgG was incubated with SWCNTs 30 min prior to MPO addition. Then, the obtained samples were used to assessment of SWCNTs degradation by Raman spectroscopy and transmission electron microscopy (TEM) [6,8,9].

2.4. Molecular modeling, docking of SWCNTs to IgG

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 IgG-ray crystal structure (PDB ID: 1IGT) by AutoDock software, as described previously [6,8].

2.5. Release of MPO and OCl^- generation in neutrophils

Cells were incubated with either serum-opsonized zymosan (SOZ) or nanotubes for 30 min. Then, neutrophils were centrifuged and the obtained supernatant was used for MPO, OCl^-, O_2^- and H_2O_2 measurements according to previous studies [6,8].

3. Results and discussion

3.1. The interactions of IgG with SWCNTs

Molecular docking studies were first performed using AutoDock software to structurally characterize possible SWCNTs interaction sites on IgG. The predicted best interaction site with lowest binding energy (−11.93 Kcal/mol) revealed that the strong binding was located in the region of the F_c fragment in IgG (Fig. 1A). GLN287, THR303, GLN305, GLN307, THR308, ARG310 and LYS345 were located in the binding pocket (Fig. 1B) and predicted to stabilize the interaction between SWCNTs and IgG.

Firstly, the oxidized groups (carboxyl) on SWCNTs in the binding site were stabilized by electrostatic interaction with these positively charged residues, ARG310 and LYS345 on IgG (Fig. 1B). The electrostatic interactions with SWCNTs were similarly observed for HSA and MPO, and the interactions were related to the positively charged Arg residues (such as Arg 160 for HSA [8], Arg 294, Arg 307, Arg 507 for MPO [6]). Our simulations revealed that polar residues, e.g., three glutamines (GLN287, GLN305, GLN307) and two threonines (THR303, THR308) in F_c domain, seemed to have significant contributions to the strong binding of proteins onto CNTs (Fig. 1B). Based on previous studies [16,17] and results herein, it could be demonstrated that these hydrophilic residues contacted SWCNT 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–CNT binding. Therefore, these computer simulation results herein illustrated that the electrostatic interaction of positively charged residues (ARG, LYS) with carboxyls on SWCNTs and hydrophobic interactions between SWCNTs and polar residues (GLN, THR) in IgG might be the crucial factors in stabilizing the binding of carboxylated SWCNTs with IgG.

To confirm the findings mentioned above, we attempted to semi-quantitatively analyze protein adsorption by SDS-PAGE and quantify the binding of IgG to SWCNTs (Fig. S1). Adsorbed proteins released from SWCNT were analyzed in Fig. S1A. After 10 min of incubation, the band intensity of protein rapidly changed from light to dark, which indicated that protein content in the sediment became much bigger due to protein adsorption onto SWCNT molecules. The protein-binding capacity of SWCNTs at 60 min was 0.52 ± 0.01 mg IgG 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]. These different protein-binding capacities were possibly related to the characteristics of protein and the different numbers of protein molecules on top of SWCNTs. The characteristic folds in IgG are grouped together in different segments, two identical F_{ab} segments connected via the hinge region to one F_c segment, thus forming a Y-shaped conformation [22]. IgG exhibiting considerable segmental flexibility from Y-shaped structure was more accessible to binding onto SWCNT surface. Although the similar molecular weights were present for IgG (~148 kDa) and MPO (~144 kDa), the numbers of each protein binding to SWCNT surfaces were about three and two molecules on average for Ig [16], and