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Characterization of the interactions between protein and carbon black

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HIGHLIGHTS

- BSA and BALF were used to investigate protein and CB interaction.
- Alteration in physicochemistry of CB was induced by BSA.
- Enzyme modulators and hydrolase predominately interacted with CB.
- Protein-to-CB interactions were associated with the coagulation pathways.
- Acute-phase response could be activated by protein–CB conjugates.

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ABSTRACT

A considerable amount of studies have been conducted to investigate the interactions of biological fluids with nanoparticle surfaces, which exhibit a high affinity for proteins and particles. However, the mechanisms underlying these interactions have not been elucidated, particularly as they relate to human health. Using bovine serum albumin (BSA) and mice bronchoalveolar lavage fluid (BALF) as models for protein–particle conjugates, we characterized the physicochemical modifications of carbon blacks (CB) with 23 nm or 65 nm in diameter after protein treatment. Adsorbed BALF-containing proteins were quantified and identified by pathways, biological analyses and protein classification. Significant modifications of the physicochemistry of CB were induced by the addition of BSA. Enzyme modulators and hydrolase predominately interacted with CB, with protein-to-CB interactions that were associated with the coagulation pathways. Additionally, our results revealed that an acute-phase response could be activated by

Abbreviations: BSA, bovine serum albumin; BALF, bronchoalveolar lavage fluid; CB, carbon black; DAVID, Database for Annotation, Visualization and Integrated Discovery; d-H_2O, distilled water; EDX, energy dispersive X-ray microanalysis; PANTHER, Protein ANalysis THrough Evolutionary Relationships; SEM, scanning electron microscopy; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gels.

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these proteins. With regard to human health, the present study revealed that the CB can react with proteins (~55 kDa and 70 kDa) after inhalation and may modify the functional structures of lung proteins, leading to the activation of acute-inflammatory responses in the lungs.

1. Introduction

The respiratory system is one of the most common routes by which particles enter the human body. Generally, particles less than 2.5 μm in aerodynamic diameter can be inhaled into the alveolar space without being removed from the respiratory tract by the mucociliary escalator, especially particles that are less than 100 nm in diameter (also known as nanoparticles) [1]. Inhaled nanoparticles first make contact with the fluid surfactant layer lining the alveoli, which consists of surfactant lipids, proteins and antioxidants. Depending on the nature of the nanoparticles, reactive oxygen species may be generated, thus disturbing the oxidative balance in the lung environment. The toxicity of the particle may be mitigated by components of the epithelial lining fluid, such as antioxidants. The presence of proteins in the lining fluid could also resolve the biological effects of particle exposure. However, the mechanisms of protein–particle interaction remain unclear.

Numerous studies have focused on the biomedical applications of nanoparticles because of their distinct physicochemical characteristics [2]. The applications of nanoparticles are significantly hampered by the possibility of adverse human health effects after exposure. For example, oxidative–inflammatory reactions and DNA damage are induced by exposure to nanoparticles such as carbon black (CB) [3,4]. Furthermore, serum proteins such as bovine serum albumin (BSA) are commonly used in toxicology experiments. For example, the addition of serum protein, such as BSA, acts as an excellent dispersant for zinc oxide nanoparticles in biological solutions [5,6]. Nanoparticles that are introduced into biological systems can follow different routes before reaching the eventual target [7]. During particle transportation in biological environments, it is important to understand the interactions of biological components with particles at the molecular level. Macromolecules such as proteins may play an important role in the binding to nanoparticles due to the forces arising from the physicochemical properties of particles [8,9]. Particle surface interactions determine their bondability with proteins as well as their removal by clearance, uptake and trafficking processes. Previous studies have been conducted to characterize protein–particle conjugates [8,10–13]. However, the potential mechanisms underlying interactions between protein and CB remain unclear.

The interactions between nanoparticles and surfactant proteins or BSA have been investigated. It suggested that there could be different adsorption mechanisms when nanoparticles interacted with different proteins [14]. Pulmonary surfactants adsorbed on nanoparticles are associated with oxidative–inflammatory potential [15]. However, few studies have identified the potential health hazards of proteins bound to nanoparticles at the biomolecular level. The objective of this study was to characterize the interactions of proteins with CB. To achieve this objective, the physicochemical characteristics of CB was assessed, as were their zeta potential, hydrodynamic diameters, structural properties, elemental characteristics and aggregation status. The bondability of protein–particle conjugates was examined, and particles bound to proteins from the bronchoalveolar lavage fluid (BALF) were identified. Finally, we analyzed the biological processes, protein classification and pathways based on the findings of protein–CB conjugates.

2. Materials and methods

2.1. Particle and reagent sources

The sources and physical properties of all hydrophobic CB (>95% carbon) used in this study are listed in Table 1. All of the other reagents were obtained from Sigma (USA) if the source is not explicitly stated.

2.2. Characterization of particles

Particle solutions were freshly prepared at 1 mg/mL in distilled water (d-H_2O) or bovine serum albumin (BSA; 5%), vortexed and sonicated for 10 min. The zeta potential of these samples (including the BSA and d-H_2O controls) was determined using a Zetasizer (Nano Z; Malvern, UK). The hydrodynamic diameters of the particles were determined using a NanoSight (LM-10; Amesbury, UK). The principle behind NanoSight has been previously reported [17]. The samples were run on the NanoSight using Nanoparticle Tracking Analysis software version 2.3 in the automatic/basic mode using the same settings for the camera shutter/gain, detection threshold brightness and gain prior to 1 min sonication. Flow cytometry (BD Biosciences, USA) was used to quantify the changes in the size population of these particles before and after BSA treatment, with 10,000 particles per event recorded for each sample. Suspending the particles in a stream of fluid and passing them by an electronic detection apparatus allowed physical characteristics of the particles to be analyzed.

2.3. Field emission-scanning electron microscopy and energy dispersive X-ray microanalysis

Pelleted samples were obtained by centrifugation at 1200 rpm and washed thoroughly by repeated (three times) resuspension and centrifugation in d-H_2O. The samples were then lyophilized for scanning electron microscope (SEM) analysis. The samples were adhered on 12-mm carbon sticky tabs, which were fixed on 13-mm aluminium SEM stubs [18]. The samples were coated with platinum to an average thickness of 10 nm using a sputter coater and then imaged. The morphologies of the samples were observed using an Inspect^{TM} SEM (FEI, USA) at an accelerating voltage of 15 kV with a 2.5 spot size. X-ray microanalysis was performed using the energy dispersive X-ray (EDX) Genesis Microanalysis System.

2.4. Particle and protein binding assay

To investigate the protein–particle conjugates, fresh BSA and bronchoalveolar lavage fluid (BALF) were used to determine the ability of the proteins to bind to particles and identify the types of BALF proteins that bound to particles. BALF samples were obtained from 8-week-old healthy female BALB/c mice (BiOLASCO, Taipei, Taiwan). The animals were maintained at a consistent temperature and relative humidity of $22 \pm 2\,^{\circ}C$ and $55 \pm 10\%$, respectively, on a 12-h light/dark cycle. All animal experiments were performed in compliance with the animal and ethics review committee of