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Conformational changes in human plasma proteins induced by metal oxide nanoparticles

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ABSTRACT

The interaction of nanoparticles (Nps) with body fluids may induce conformational changes in the proteins present in the medium. Such interactions could induce functional loss or important modifications in some proteins, and trigger cellular events induced by the Np-protein moiety. As metal oxide nanoparticles are widely used for various applications, the interaction of four different metal oxide Nps (ZnO, TiO_2, CeO_2 and Al_2O_3) with three of the main protein fractions from human plasma (albumin, fibrinogen and globulins) was characterized by fluorescence and Fourier-transform infrared (FTIR) spectroscopy. The pattern of Np–protein interaction was shown to vary depending on the type of Np. For ZnO Nps, a strong interaction was observed, which induced a decrease in the thermal stability of both fibrinogen and albumin at a low temperature, interfering with the clotting activity of fibrinogen. TiO_2 and CeO_2 Nps showed lower effects, while for Al_2O_3 Nps only a slight or null interaction was observed at physiological pH. Moreover, the influence of pH was characterized for albumin, showing that the Np–protein interaction has an important dependence on the Np surface charge.

The conformational changes induced by metal oxide Nps in the secondary structure of albumin are principally the transformation of α-helices into β-sheet structures. The interaction, with the exception of Al_2O_3 nanoparticles at basic pH, could take place in the domain II of the protein, formed mainly by hydrophobic and positive residues.

1. Introduction

The potential toxicity of nanomaterials (NMs) has raised many concerns in the nanotechnology research field and in the regulatory/advisory committees.

Metal oxide nanoparticles (Nps), such as TiO_2, ZnO, CeO_2 and Al_2O_3 Nps are now being produced in large quantities and commercialized for several purposes. For example, titanium dioxide and zinc oxide Nps are widely used in cosmetic products and they could be used as sterilizing agents due to their photocatalytic function [1]. Although ZnO Nps are not able to penetrate the *stratum corneum* and to access the epidermis, (apparently being safe for topic applications), they have been shown to induce toxicity in prokaryotic and eukaryotic cells and in zebrafish embryos [2–5].

CeO_2 and Al_2O_3 Nps are used for different physicochemical applications, such as chemical–mechanical polishing and catalysis [6,7]. These nanoparticles have shown their capacity to neutralize free radicals in cells, preventing inflammation and demonstrating neuroprotective effects [8,9].

When administered *in vivo*, nanomaterials have a great tendency to bind to fluid and body proteins. The proteins bound to the NMs surface constitute the so called protein corona, and this differs depending on the type of NM, as well as the size and charge, among other factors [10].

Human plasma represents about 50% of the total blood volume, and it is composed of many proteins such as albumin (the most abundant protein), fibrinogen, gammaglobulins, complement factors, and α1-antitrypsin. When administered intravenously, many plasma proteins form the protein corona around the NMs. Albumin, fibrinogen, apolipoproteins, immunoglobulins and complement factor C3b are the most common plasma proteins found attached to different NMs [11].

The protein corona has been extensively characterized by several authors [11–16], having a considerable influence on the physicochemical characteristics of a NM, on its interaction with body components and on its biodistribution.

The plasma proteins found attached to the surface of diverse nanomaterials vary according to the physicochemical properties of the nanostructure, with surface charge and hydrophobicity being

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the most relevant factors, whereas size and surface curvature only have an influence on the amount (but not on the type) of protein bound [11]. Hydrophobic particles usually bind more serum proteins than hydrophilic particles of the same nature [14]. Surface charge increases the amount of bound protein, but has little influence on the type of protein attached [15]. Nevertheless, the sign of the charge strongly influences the pattern of the bound protein. Positively charged polymeric Nps preferentially absorb proteins with isoelectric points lower than 5.5 and, in contrast, negatively charged Nps preferentially interact with proteins with an isoelectric point higher than 5.5 [16]. Interestingly, in most cases, the surface coating of the NMs is also irrelevant for the identity of the bound protein, even when the coating may reduce the amount of protein attached to the NMs [17–20].

The interaction of serum proteins with the NMs can have negative consequences, such as the induction of conformational changes. Protein conformational changes may induce the loss of protein functionality, the modification of the interaction with other elements, or even the initiation of inflammatory/allergic or autoimmune responses. Moreover, pH changes may also have an influence on protein conformation and on protein–NM interaction. Conformational changes in plasma proteins induced by metal oxide Nps have not been extensively characterized, although it has been reported that some nanomaterials can induce protein changes [21,22]. The morphology and the charge of the NMs seem to strongly influence the interaction with the proteins [22].

It has recently been shown that the interaction of negatively surface charged gold Nps with human plasma induces the unfolding of fibrinogen as revealed by circular dichroism [23]. Consequently, an inflammatory response was induced *via* the interaction of the unfolded fibrinogen with the Mac-1 receptor on the surface of a monocyte cell line (THP-1), and the activation of the NF-κB signaling pathway of these immune cells.

Another recent study has described how the interaction between supermagnetic iron oxide nanoparticles (SPIONs) and human transferrin induces irreversible conformational changes with the release of iron, which alters the main function of the protein. This change in the transferrin conformation from a compact to an open structure is not reversible even after the removal of the nanoparticle [24].

In contrast, some Np–protein interactions induce positive effects, such as enzymatic stabilization (decreasing degradation). For example, adsorption of lipases on nanostructured polystyrene (PS) and polymethylmethacrylate (PMMA) leads to increased performance in terms of enzymatic activity and selectivity, as well as an increased thermal stability, when compared to lipases on non-nanostructured counterparts [25].

To study structural changes in plasma proteins in the presence of metal oxide Nps, three main human plasma fractions (fibrinogen, albumin and globulins) were isolated and then incubated in the presence of uncoated zinc, cerium, titanium and aluminum oxide Nps. Fluorescence spectroscopy was used to analyze the Np–protein interaction. Tryptophan (Trp) fluorescence is very sensitive to the structural changes in the protein induced by interaction with the solvent or other molecules. Trp fluorescence emission spectra have a maximum at around 335 nm, characteristic of the amino acid located in the hydrophobic core. However, Trp exposed to the solvent shows a maximum at around 350 nm [26].

As fibrinogen contains 11–14 Trps in every chain, changes in the fluorescence cannot be attributed to a single chain, but to the whole protein [27]. In the case of globulins, they include a large variety of proteins and their fluorescence signal is intense, which is indicative of a high Trp content [28].

In contrast, human serum albumin only has one Trp residue (Trp214) at subdomain IIA, which allows the assignment of the spectral changes to this protein region [29]. The protein also has 18 tyrosines and 31 phenylalanines, but their fluorescence emission is much lower than that emitted by the Trp. Moreover, if the protein is excited at 290 nm, the changes observed in the spectra correspond mainly to the Trp environment, with a small contribution from the tyrosines and phenylalanines. Finally, the influence of the pH, Np zeta potential and protein charge was also characterized.

In the present study, commercial human serum albumin (HSA) was used to confirm the protein–Np interaction detected with the albumin fraction obtained from human plasma. Fourier-transform infrared spectroscopy (FTIR) measurements were also taken to further characterize the conformational changes in albumin detected by fluorescence spectroscopy. FTIR spectroscopy is a technique widely used for studying protein conformational changes, and mainly used to characterize changes in secondary structure [30].

2. Experimental

2.1. Human plasma protein fractioning

Three plasma protein fractions (fibrinogen, globulins and albumin) were separated by the salting out method using ammonium sulfate as precipitating agent. An aliquot of 0.5 ml of human plasma from a healthy donor was suspended in Tris/EDTA (10 mM, pH 7.4, 3.5 ml). Ammonium sulfate was added at 20%, 60% and 70% at 4 °C to precipitate fibrinogen, globulins and albumin, respectively. Every precipitated fraction was collected by centrifugation (10,000 × *g* at 4 °C for 15 min) in a microcentrifuge, Eppendorf 5415R, suspended in 1 ml of PBS pH 7.4 and exhaustively dialyzed against PBS in dialysis cassettes with a membrane of 10 kDa MWCO (Thermo Scientific Inc., Slide-A-Lyzer Dialysis Cassettes). An electrophoresis was performed in a 10% SDS-PAGE gel to check protein purity. The gel was stained with Bio-Safe Coomassie G-250 Stain from Bio-Rad (cat. number 161-0786). Protein fractions where kept at −20 °C and thawed at 4 °C prior to their use for fluorescence spectroscopy studies.

2.2. Nanoparticles

Different uncoated metal oxide nanoparticles were tested: TiO_2 Nps (3.59 ± 0.94 nm), and Al_2O_3 Nps (13.56 ± 8.37 nm) were supplied by PlasmaChem (Berlin, Germany). CeO_2 Nps (13.04 ± 12.13 nm) and ZnO Nps (36.16 ± 18.27 nm) were supplied by Evonik Industries AG (Essen, Germany). Np size was determined by transmission electron microscopy (TEM) in the laboratory of Dr. Sergio Moya (CIC Biomagune, San Sebastian, Spain). All Nps were suspended in Milli-Q water (10 mg/ml) and sonicated in an ultrasound bath (Branson 1510, Danbury, CT), at low frequency (47 kHz) for 10 min.

2.3. Zeta potential and size measurement

Measurement of the zeta potential and size of the Nps was performed using a Zetasizer Nano ZS from Malvern instruments at 25 °C. Suspensions of Nps at 1 mg/ml in water or 5 mM Hepes buffer at different pH values were prepared for the zeta potential determination. Each sample was measured 4 times, combining 200 runs per measurement. For zeta size determination, Nps (25 μg/ml) were suspended in water, PBS or albumin (400 μg/ml) and 3 measurements were averaged, combining 13 runs per measurement. The Np size distribution was determined by intensity.

2.4. Fluorescence spectroscopy

Fluorescence measurements were taken on a FluoroMax-3 fluorimeter (HORIBA Jobin Yvon Inc.) at 25 °C using a thermostatic