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A quantitative binding study of fibrinogen and human serum albumin to metal oxide nanoparticles by surface plasmon resonance

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ABSTRACT

The interaction of plasma proteins with metal oxide nanoparticles (NPs) is important due to the potential biomedical application of these NPs. In this study, new approaches were applied to measure quantitatively the kinetics and affinities of fibrinogen and human serum albumin (HSA) for TiO_2, CeO_2, Al_2O_3 and ZnO NPs immobilized on a sensor chip. Real-time surface plasmon resonance (SPR) measurements showed that fibrinogen interacted with TiO_2 and CeO_2 NPs with high affinity (135 and 40 pM, respectively) and to Al_2O_3 NPs with moderate affinity (15 nM). The data fitted well to the Langmuir model describing a 1:1 interaction. In contrast, HSA interacted with TiO_2, CeO_2 and Al_2O_3 NPs with lower affinity (80 nM, 37 nM and 2 μM, respectively) with the data fitting better to the conformational change model. TiO_2 and CeO_2 NPs had fast association rate constants with fibrinogen ($1 \times 10^6\ M^{-1}\ s^{-1}$) and Al_2O_3 NPs had a slower association rate constant ($1 \times 10^4\ M^{-1}\ s^{-1}$). By contrast, HSA had markedly slower association rate constants (1×10^3–$1 \times 10^4\ M^{-1}\ s^{-1}$). The binding of the proteins was reversible, thus allowing the rapid capture of data for replicates. The occurrence of matrix effects was evaluated by using surfaces with different chemistries to capture the NPs, namely alginate, NeutrAvidin and bare gold. The affinity values determined for the NP–protein interactions were largely independent of the underlying surface used to capture the NPs.

1. Introduction

The binding of proteins to different inorganic surfaces plays an important role in natural processes such as the growth of bone tissue (protein–mineral interactions), in biotechnological applications and in the medical implant sector (Lynch et al., 2007; Monti et al., 2008). However, the mechanism for the interaction between proteins and inorganic surfaces is complex and is still not fully understood (Nakanishi et al., 2001).

Recently, the binding of proteins to metal oxide surfaces has received increasing attention due to the important role that this phenomenon plays in determining the biocompatibility of metal oxide materials for use in medicine (Costa et al., 2013; Kang et al., 2010) as well as in the preparation of new biosensors. For example, the protein layer deposited on the surface of surgical metal implants can induce early counterproductive immunological responses in patients (Monti et al., 2007; Vallee et al., 2010). The physico-chemical properties of the NPs, such as composition, size, charge and surface coating, can dramatically influence their interaction with proteins (Mahmoudi et al., 2011) and thus their possible future uses. To understand this binding fully it is necessary to have knowledge not only of which proteins are attached to the particles, but also their association and dissociation rates, affinity and the stoichiometry of the complexes (Aggarwal et al., 2009). Several established techniques have been used to analyze some of these parameters, including size-exclusion chromatography (SEC), isothermal titration calorimetry (ITC), quartz crystal microbalance (QCM) and SPR (Cedervall et al., 2007; Klein, 2007).

SPR is a label-free technique that was introduced in the 1990s to study interactions between biomolecules in real time and it was mainly employed for antibody–antigen complexes (Liu et al., 2006). In the last two decades, SPR has been extended to other areas such as drug discovery and development, environmental protection, food analysis, medical diagnostics, immunogenicity and NP–protein interactions (Huber and Mueller, 2006; Karlsson, 2004; Xu et al., 2010). This technique provides the reaction

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kinetics and affinity constants of molecular interactions as well as the active concentration of bio-molecules in solution (Homola, 2008). SPR has been used to measure the interaction of fibrinogen, albumin and even whole plasma with two polymeric NPs. In that work, the NPs were immobilized on the gold surface by chemical modification of the NPs with thiol groups (Cedervall et al., 2007). However, chemical modification could be a limiting factor in the study of some NPs and the protein binding affinity could be affected by NP surface modification. The direct capture of unmodified NPs on the surface of the SPR chips will allow a more realistic scenario for the analysis of the interaction between NPs and proteins and this may help to predict the behavior of NPs in body fluids. To our knowledge, only Cohavi et al. (2011) have described the direct immobilization onto an SPR chip surface and they immobilized gold NPs onto a NeutrAvidin chip surface to study the binding with peptides and proteins. In the work presented here we extended this study to a range of different, more medically relevant, metal NPs.

In the present work, the binding of fibrinogen and HSA to different metal oxide NPs, namely TiO_2, CeO_2, Al_2O_3 and ZnO, was evaluated by SPR using the ProteOn™ XPR36 Protein Interaction Array System (Bio-Rad). The kinetic binding constants and affinities of the interactions between NPs immobilized on the surface of the chip and proteins passed over them as analytes were studied with the aim of comparing the results for both proteins and each type of metal oxide NP used. Three sensor chips with different chemical surfaces [compact alginate (GLC), NeutrAvidin (NLC) and bare gold (BGD)] were used to capture the NPs in order to examine whether the capture chemistry had an effect on the interaction. For the interaction of HSA with Al_2O_3 NPs, the high capacity alginate sensor chip (GLH) was also used to increase the amount of NPs captured. For ZnO NPs, the interaction was studied by immobilization of the NPs and injection of the proteins as analytes and in reverse by immobilization of the proteins followed by the injection of the ZnO NPs as analytes.

2. Materials and methods

2.1. Reagents

GLC, GLH and NLC chips and SDS were purchased from Bio-Rad (Bio-Rad Laboratories Inc.) and PBS buffer reagents (KCl, NaCl, KH_2PO_4, Na_2HPO_4 and Tween-20) were purchased from Thermo Fisher (Thermo Fisher Scientific Inc.).

2.2. Human serum proteins

Human serum albumin (HSA) ($\geq$96%) and fibrinogen were both purchased from Sigma-Aldrich (LLC, St. Louis, MO). Fibrinogen had a purity of 50–70%, with approximately 15% of sodium citrate and 25% of sodium chloride. The percentage of clottable protein was $\geq$80% from the total amount of fibrinogen.

2.3. Nanoparticles

Four uncoated metal oxide nanoparticles were tested: TiO_2 NPs (3.59 ± 0.94 nm) and Al_2O_3 NPs (13.56 ± 8.37 nm), supplied by PlasmaChem (Berlin, Germany), and CeO_2 NPs (13.04 ± 12.13 nm) and ZnO NPs (36.16 ± 18.27 nm), 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 (Selecta 300838, Ultrasons-H) at low frequency (40 kHz) for 20 min.

The Dynamic Light Scattering (DLS) characterization of the NP size and the *Z*-potential measurements were performed using a Zetasizer Nano ZS from Malvern instruments at 25 °C. Suspensions of NPs at 1 mg/ml in water were prepared for the Z-potential determination. Each sample was measured 4 times, combining 200 runs per each one.

For NP size distribution analysis, NPs (25 μg/ml) were suspended in water or PBS buffer and 3 measurements were averaged, combining 13 runs per each one. The NP size distribution was determined by intensity. The size and Z-potential characterization of the NPs are summarized in Table S6.

2.4. Immobilization of TiO_2, CeO_2, Al_2O_3 and ZnO NPs on different commercial sensor chips

For further details, please see Supplementary information.

TiO_2, CeO_2, Al_2O_3 and ZnO NPs were directly immobilized by physisorption (Elaissari, 2008) on different commercial SPR sensor chips used in the ProteOn™ XPR36 instrument (Bio-Rad). TiO_2, CeO_2 and Al_2O_3 NPs bind irreversibly with a high response (in Response Units, RU) to alginate and NeutrAvidin surfaces. ZnO NPs bind to the surfaces reversibly.

TiO_2, CeO_2, Al_2O_3 and ZnO NPs were applied at the concentrations described (Supplementary information) to different flow cells in Milli-Q water at a flow rate of 25 μL/min until an appropriate signal was reached. Another flow cell was left blank to serve as a reference channel (Fig. 2). Four different sensor chips were used: GLC, GLH, NLC and BGD.

In the case of the BGD sensor chip, which has a low level of charge and does not contain functional groups on the surface, the NPs were directly immobilized, giving a high response. However, after the protein injection, the signals presented drift and distortion, and decreased in the consecutive sensorgrams, probably due to a dissociation of the NPs from the surface. For that reason, the direct immobilization of the TiO_2 and CeO_2 NPs on the chip produced an unstable surface so a different strategy was used for the immobilization of these NPs. A stable surface of Al_2O_3 NPs (an inert surface for fibrinogen and HSA at the protein concentration injected) was used as an intermediate surface, to which the NPs could be easily immobilized (Fig. 1).

The Al_2O_3 NPs, at a concentration of 100 μg/mL in Milli-Q water, were applied at a flow rate of 25 μL/ min until a signal of about 3000 RU was reached in the three flow cells. After injection, Milli-Q water was passed through the system for 12 h until a stable baseline was achieved. The TiO_2 and CeO_2 NPs, at the concentrations described (Supplementary information) in Milli-Q water, were then applied at the same flow rate until an appropriate signal was reached in two flow cells. The Al_2O_3 flow cell was used as a reference to subtract possible interferences. The signals were obtained by subtracting the signal observed on the Al_2O_3 NP channel from those of the two flow cells with the TiO_2 or CeO_2 NP channels. For the interaction with fibrinogen, the Al_2O_3 NP reference channel was 200 RU lower than that on the TiO_2 NP channel, and 215 RU lower than the CeO_2 NP channel. For the interaction with HSA, the Al_2O_3 NP reference channel was 50 and 66 RU lower than those of the TiO_2 and CeO_2 NP channels, respectively.

2.5. Protein injection and kinetic analysis

The interaction analyses with fibrinogen and HSA were conducted at 25 °C in PBST (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.005% Tween 20, pH 7.4) unless stated otherwise.

After the immobilization of the NPs, the buffer was changed to PBST. The chip was then rotated by 90° and buffer injections were performed until a stable signal was achieved (for at least 30 min).