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Controlling protein–particle adsorption by surface tailoring colloidal alumina particles with sulfonate groups

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ABSTRACT

In this study, we demonstrate the control of protein adsorption by tailoring the sulfonate group density on the surface of colloidal alumina particles. The colloidal alumina (d_{50} = 179 ± 8 nm) is first accurately functionalized with sulfonate groups (SO_3H) in densities ranging from 0 to 4.7 SO_3H nm^{-2}. The zeta potential, hydrophilic/hydrophobic properties, particle size, morphology, surface area and elemental composition of the functionalized particles are assessed. The adsorption of three model proteins, bovine serum albumin (BSA), lysozyme (LSZ) and trypsin (TRY), is then investigated at pH 6.9 ± 0.3 and an ionic strength of 3 mM. Solution depletion and zeta potential experiments show that BSA, LSZ and TRY adsorption is strongly affected by the SO_3H surface density rather than by the net zeta potential of the particles. A direct correlation between the SO_3H surface density, the intrinsic protein amino acid composition and protein adsorption is observed. Thus a continuous adjustment of the protein adsorption amount can be achieved between almost no coverage and a theoretical monolayer by varying the density of SO_3H groups on the particle surface. These findings enable a deeper understanding of protein–particle interactions and, moreover, support the design and engineering of materials for specific biotechnology, environmental technology or nanomedicine applications.

1. Introduction

Inorganic colloidal particles are versatile for use in biomedical and biotechnological applications, e.g. as drug carriers, for cell targeting or protein purification, as adsorbents or as biosensors [1,2]. When in contact with biological environments, proteins immediately coat the particles. A protein coating significantly affects the functions and fate of sub-micron biomaterials, along with the biological response to colloids, i.e. their biological transport paths, targeting and sensing ability, cellular uptake, and toxic responses [3–5]. Fundamental techniques to control the multifaceted processes of non-specific protein–particle adsorption remain unclear, but are highly sought after as ways to develop advanced biomaterials [6]. Surface functionalization strategies that control protein adsorption may advance several applications in which specific amounts of proteins have to be deposited on a particle surface or removed from solution, including protein separation/purification technologies, drug carrying, chromatography and biosensors.

It is becoming apparent that particle surface properties effectively influence the assembly of the so-called "protein corona" around a particle [7,8]. Tailored surface functionalizations potentially direct the protein–particle interactions [5,9,10]. Hydrophobic surface functionalizations, i.e. with alkyl groups, increase the adsorption of proteins by decreasing the energy needed for the displacement of surface water molecules with proteins [11–14]. Functionalization with hydrophilic molecules like poly(ethylene glycol) reduces protein adsorption [5]. Charged groups like carboxyl, amino, sulfonate and phosphate groups affect the protein adsorption due to electrostatic interactions [8,15–21]. However, protein adsorption is not always simply a matter of attraction between oppositely charged proteins and particles. Protein–particle adsorption can deviate greatly from predictions made from the net charges and isoelectric point (IEP) of the protein and the particle [8,19]. When a particle is transported from one biological milieu into another, the formation of the protein layer and the reversibility and reformation of the protein corona are influenced by the particle's surface chemistry [22]. Milani et al. [23] showed that preadsorbed transferrin is completely removed from carboxylated particles when exposed to plasma but remains on sulfonated particles, although both functional groups are net negatively charged.

As well as the type of the functional groups, their surface density, defined as amount of functional groups per surface area, is an important parameter. Protein adsorption is strongly influenced by the surface density of the functional groups, their local distribution on the particle surface and the influences of the substrate surface [24]. Comparative studies that investigate the influence of

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these parameters on protein–particle adsorption are rare and the mechanisms involved remain unclear. Nevertheless, a direct correlation between the surface density of functional groups and the protein adsorption behavior could be a potential tool to optimize the surface functionalization of colloids with applications in biological systems.

Here, we investigate protein adsorption as a function of fine-tailored surface densities of sulfonate groups on colloidal alumina particles. Alumina is widely used as a substrate material for copious biomedical and biotechnological applications, e.g. in protein separation and purification systems [25], biosensors [25], biocatalysis [26,27] or drug carrier applications [28]. Surface functionalization with sulfonic acids is often used to stabilize inorganic particles in biological media, or to introduce ion exchange [29] or catalytic properties [30]. Additionally, sulfonic acids (e.g. taurine) are involved in many biochemical reactions and can bind to proteins [31]. The combination of alumina and tailored SO_3H surface functionalization is promising for several environmental, biotechnolocial and biomedical applications, particularly for protein purification, protein separation and chromatography, but is nevertheless rarely studied.

The native alumina particles feature amphoteric Al–OH groups on the surface that are mainly protonated below the IEP of alumina at pH ~9.3, resulting in a positive charge. In contrast, SO_3H groups are deprotonated and strongly negatively charged above pH ~1.5 due to their acid dissociation constant (pK_a) of 1.53 (the pK_a of propyl sulfonic acid) [32]. In a previous study, we showed that the introduction of a monolayer of SO_3H groups on colloidal alumina (and also carboxyl, phosphate and amino groups) strongly changes the protein adsorption pattern and reduces the adsorption of negatively charged proteins, though it promotes adsorption of positively charged proteins by providing sites for electrostatic repulsive or attractive protein–particle interactions [20]. In this study, by accurately controlling the SO_3H surface density, particles are synthesized that are either maximally covered with SO_3H or are only partially functionalized. This introduces diverse potential protein interaction sites and allows for the adjustment of surface charges within a broad range.

Bovine serum albumin (BSA), lysozyme (LSZ) and trypsin (TRY) were selected to study protein adsorption due to their well-known properties, their relevance as model systems, and their presence in biological and body fluids [33]. BSA, LSZ and TRY each exhibit a specific amino acid composition, size, structure and IEP. The protein adsorption experiments were conducted under defined and constant media conditions (pH 6.9 ± 0.3 and ionic strength of 3 mM). These conditions were chosen to accentuate the interrelation between the specific particle surface functions and the individual protein properties. We used the constant pH and avoided high ionic strengths to highlight the charge differences between particles with different SO_3H surface density. Additionally, the proteins were used separately. Interfering protein–protein or other overlaying interactions occurring in biological milieus such as blood, containing many other proteins and ions, were thereby avoided and the influences of the particle surface chemistry therefore remained in focus [9,34].

The protein–particle adsorption was determined by comparing the protein solution depletion and the zeta potential of the protein–particle complexes. An empirical approach to estimate the zeta potential of protein–particle complexes is the correlation between the degree of particle surface covered by proteins and the zeta potential of the particles and proteins separately [35,36]. This approach was employed to prove how surface functional groups and adsorbed proteins contribute to the zeta potential of protein–particle complexes.

2. Materials and methods

2.1. Materials

Polycrystalline α-alumina particles (TM-DAR, Lot. 8086, high purity alumina >99.99%, d_{50} = 179 ± 8 nm, density = 3.98 g cm^{-2}, specific surface area = 11.5 m^2 g^{-1}; Krahn Chemie GmbH, Germany) were used as received. For SO_3H surface functionalization, 3-(trihydroxysilyl)-1-propanesulfonic acid (30–35% in water, Lot. 1159776, ABCR GmbH, Germany) was used. BSA (98% purity, MW = 66.5 kDa, Lot. 040M1649, Sigma-Aldrich, Germany), LSZ from hen egg white (94% purity, MW = 14.7 kDa, Lot. 088K1358, Sigma-Aldrich, Germany) and TRY from porcine pancreas (97% purity, MW = 23.3 kDa, Lot. 089K7358, Sigma-Aldrich, Germany) were used to study protein adsorption. HCl, KOH and KCl were purchased from Sigma-Aldrich, Germany. Double deionized water with a conductivity of 0.04 μS cm^{-1} was obtained from a Synergy® apparatus (Millipore, Germany) and used in all experiments.

2.2. Surface functionalization of α-alumina particles

Alumina particles were functionalized in an aqueous process as described in Ref. [20]. In short, suspensions of α-alumina particles were prepared by mixing 15 g of particles with 50 ml of water followed by sonication for 10 min with a Sonifier® 450 ultrasound horn (Branson, USA, output: 150, pulse rate: 0.5 s) to break any agglomerates before the addition of the precursor. 3-(Trihydroxysilyl)-1-propanesulfonic acid was mixed with 50 ml of water to final concentrations between 0 and maximal 0.102 M, corresponding to 29.6 μmol per m^2 of the specific surface area of the unmodified α-alumina particles. The precursor solution was added to the particle suspensions and stirred for 60 min at 25 °C and was then heated for 90 min at 115 °C. Afterwards the particles were separated by centrifugation and washed three times in 100 ml of water to remove any unreacted precursor. Particles were freeze-dried for 96 h at −20 °C in a vacuum using a P8KE-80 freeze dryer (Piatkowski Forschungsgeräte, Germany).

2.3. Particle characterization

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was carried out to determine the sulfur content of the functionalized particles in an ICAP 6500 (Mikrolabor Pascher, Thermo, Germany). Samples were analyzed after they had undergone acid pressure hydrolysis. The average and standard deviation were calculated from two measurements.

Potentiometric titration was carried out using a Titralab 840 automatic titrator (Radiometer, France). For this, 1.35 g of particles was mixed with 35 ml of water and sonicated for 10 min, then 0.5 ml of 1 M HCl was added. The particles were then back titrated with 0.1 M KOH in a nitrogen-flushed vessel to determine the total amount of protons in the sample. The amount of hydroxyl groups on alumina was determined by the difference from a reference sample without particles, as described by Campos et al. [37]. The amount of SO_3H groups was calculated from the difference between functionalized and unfunctionalized particles. Transmission electron microscopy (TEM) was carried out using a Titan 80-300 ST microscope (FEI™, the Netherlands) equipped with a Cs-corrector for the imaging lens, using a 300 kV electron beam and a vacuum at 1.3×10^{-7} mbar. Two-hundred-mesh S162 copper grids covered with a form var film (PLANO GmbH, Germany) were used as sample holders. Specific surface areas were calculated from nitrogen and argon adsorption isotherms using the Brunauer, Emmet and Teller equation [38]. Adsorption isotherms were recorded at

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