Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 4351–4357
Biomaterials
www.elsevier.com/locate/biomaterials

Lysozyme and bovine serum albumin adsorption on uncoated silica and AlOOH-coated silica particles: the influence of positively and negatively charged oxide surface coatings

Kurosch Rezwan[a,b,*], Lorenz P. Meier[a,b], Ludwig J. Gauckler[b,c]

[a]*Nonmetallic Inorganic Materials, Department Materials, ETH Zurich, Switzerland*
[b]*Nichtmetallische Werkstoffe, Wolfgang-Pauli-Str. 10, ETH Hönggerberg, HCI G 538, 8093 Zürich, Switzerland*
[c]*Nichtmetallische Werkstoffe, Wolfgang-Pauli-Str. 10, ETH Hönggerberg, HCI G 535, 8093 Zürich, Switzerland*

Received 6 September 2004; accepted 13 November 2004

Abstract

The adsorption of lysozyme and bovine serum albumin on silica and AlOOH-coated silica particles—representing negatively and positively charged oxide surfaces—was investigated. The protein-treated uncoated and completely AlOOH-coated silica particles were characterized by zeta potential analysis and UV/VIS spectroscopy. It was found that at pH 7 a protein oppositely charged to the oxide surface adsorbs in significantly higher amounts. In contrast, proteins of the same charge did not or only in very low amounts adsorbed on an oxide surface. As both oxide surfaces were measured to be very hydrophilic it can be concluded that electrostatic interactions dominate the adsorption process at the investigated experimental conditions. The pH regions where the proteins interact via attractive and repulsive coulomb interaction with the particular oxide surfaces were calculated and outlined.

Keywords: Protein adsorption; Surface charge; Silica; Alumina; Sol–gel

1. Introduction

Protein adsorption on surfaces of biomaterials and medical implants is an essential aspect of the cascade of biological reactions taking place at the interface between a synthetic material and the biological environment. Type, amount and conformation of adsorbed proteins mediate subsequent adhesion, proliferation and differentiation of cells and are believed to steer foreign body response and inflammatory processes [1–3].

Concerning the protein adsorption process, the fundamental electrostatic interactions between ceramic particles and proteins are only fragmentarily investigated and not well understood [4–10]. Especially, the influence of particle shape, size and size distribution may vary when studying the adsorption of proteins on different colloids. This can be eliminated by using a sol–gel technique to modify the surface chemistry of narrowly distributed particles. This is possible by coating well-defined silica particle surfaces using a relatively simple and cost-effective method. It is a convenient way to tailor the surface chemistry of a substrate without changing the substrate itself which can particularly be useful for e.g. biosensor applications.

The sol–gel process was used to alter the surface properties of the silica particles in order to investigate the influence of the surface charge on the adsorbed amount of lysozyme (LSZ) and bovine serum albumin (BSA). The coating process was optimized and the coating quality controlled by measuring the zeta potential and by scanning electron microscopy. The precursor used for the coating process was Al-sec-butoxide. The silica particles (negatively charged at pH 7) were coated

*Corresponding author. Nichtmetallische Werkstoffe, Wolfgang-Pauli-Str. 10, ETH Hönggerberg, HCI G 538, 8093 Zürich, Switzerland. Tel.: +41 1 632 68 53; fax: +41 1 632 11 32.
E-mail address: kurosch.rezwan@mat.ethz.ch (K. Rezwan).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.11.017

with an AlOOH layer which is positively charged at pH 7. We investigated the amounts and the resulting zeta potentials of bovine serum albumin (BSA, negatively charged at pH 7) and lysozyme (LSZ, positively charged at pH 7) adsorbed onto the negatively and positively charged oxide particles to monitor the effect of electrostatic attraction and repulsion on the amount of adsorbed proteins.

2. Experimental

2.1. Materials and reagents

2.1.1. Silica and Al-sec-butoxide

Silica Snowtex ZL (Lot. No. 140828) was obtained from Nissan Chemicals Industries Ltd. as a 30 vol% suspension. To lower the salt content, the suspension was dialyzed for a few days using a dialysis tube (ZelluTrans ROTH) till the electrical conductivity fell below 1 μS/cm. The specific surface area was characterized by X-ray disc centrifuge sedigraph and was found to be 34 m^2/g with a d_{50} particle diameter of 0.097 μm.

Al-sec-butoxide was obtained from Fluka (Lot. & Filling: 431389/143502) and was used without further modifications.

Double deionized water with an electrical resistance of 18 MΩcm from a NANOpure water system (Barnstead) was used for all experiments.

2.1.2. Proteins and protein reagent

Chicken hen egg white lysozyme (LSZ, L6876, Lot. No. 051K7028) and bovine serum albumin (BSA, A7906, Lot. No. 12K1608) were purchased from Sigma Aldrich and used without any modifications. Table 1 shows a comparison of the protein data of LSZ and BSA taken from Ref. [11,12].

Bradford reagent [13] was used as protein dye and was purchased from Sigma Aldrich (B6916, Lot. No. 52K9311) without further modifications.

2.2. AlOOH coating of the silica particles

After dialysis, the amount of silica in the suspension was 17 vol% determined by drying the suspension and by pycnometrie. From this suspension a silica suspension of 6 vol% (equal to 8 g silica in 64.4 ml H_2O) was prepared. Hereafter, the pH was adjusted to 9 by adding drops of a 24% NH_{3aq} solution. The suspension was ultrasonicated with an ultrasound horn for 5 min (UP200 s, Dr. Hielscher GmbH, 200 W) at highest performance. The de-agglomerated suspension was poured into a 250 ml two-necked round-bottomed flask and heated up to 85 °C in an oil bath while being stirred. On the flask a reflux water cooler was mounted in order to condensate the evaporating water and to minimize water losses during the coating process. After reaching a constant temperature of 85 °C, the required amount of Al-sec-butoxide was mixed with 70 ml water and poured into the flask. The different amounts used for the coatings are shown in Table 2.

Table 1
Protein data of lysozyme (LSZ) and bovine serum albumin (BSA)

Protein	Molecular weight (kDa)	Dimensions (nm^3)	Isoelectric point at pH
LSZ [11]	14.3	3 × 3 × 4.5	11
BSA [12]	66.462	5 × 5 × 5	4.7–4.9

Table 2
Different amounts of Al-sec-butoxide added to the silica suspension during the coating process

Added mass Al-sec-butoxide (g)	Al-sec-butoxide/silica (wt/wt%)	Al-sec-butoxide/silica surface area (10^{-2} g/m^2)
9.16	115	3.3
5.725	72	2.06
4.3	54	1.55
2.86	36	1.03

The Al-sec-butoxide reaction with the suspension was kept at 85 °C for 2 h under stirring conditions. Afterwards the suspension was cooled down to below 40 °C. The pH was adjusted to 3 with 2 N HCl in order to dissolve residues of amorphous AlOOH. The acidic suspension was stirred for another 10 min and subsequently ultrasonicated for 5 min. The suspension was centrifuged at 2200*g* for 30 min in a thermostatized Hermle Z 513 K. The supernatant was removed and the sediment dispersed in double-deionized water followed by an ultrasound treatment of 5 min. The wt% content was determined by drying 1 ml of the suspension. The particle density was measured by pycnometrie. Based on the vol% of silica found, water was added to dilute the obtained suspension to a 2 vol% suspension for all adsorption and zeta potential measurements. The particle size distribution was measured with an X-ray disc sedigraph (XDC, Brookhaven Instruments) and the surface tension at the air interface by the drop pendant method (PAT 1, SINTERFACE) [14].

All side products during the coating process were removed by the centrifugation step and the protein adsorption was carried out at low ionic strengths (< 1 mM KCl as determined by conductometry) and without any organic residues.

2.3. Protein addition to the suspension

We added 4.96×10^{-12} mol proteins per cm^2 silica surface area to the uncoated and coated silica suspension in