Protein interaction with SiO2 and AgNPs: from adsorption on solid surfaces to organization and conformational changes

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***Abstract*— Driven by many applications, the development of new biomaterials has considerably increased in the last decade. The current research strategies also involve revealing of the relationship between protein structure and function due to the exposure and interaction of proteins with non-biological organic and inorganic solid surfaces. Aiming at understanding of the mechanisms of protein adsorption on solid surfaces we follow in this work the organization dynamics of proteins (Bovine Serum Albumin, BSA and Fibronectin, Fn) adsorbed on thin silica layers without or with silver nanoparticles (AgNPs), deposited on their surfaces. It is found that although starting with the same protein concentration in solution (0.05 g/L), the adsorbed amount of proteins on SiO_2 surfaces is twice larger for Fn (1.32 µg/cm^2) compared to BSA (0.58 µg/cm^2). The proteins adopt different conformations according to the surface pattern. On a flat SiO_2 surface, the BSA proteins organize in a lace-like network while the Fn proteins adopt a branching-type. The patterned by AgNPs surfaces induce conformational changes of the proteins. In interaction with AgNPs both types of proteins fold up to attain mainly compact globular conformation.**

Keywords—AgNPs, SiO_2, proteins, adsorption, conformation

I. INTRODUCTION

Recent advances in biotechnology related to development of biosensors, photosensitizers, artificial implants, bioelectronics, etc. require addressing, among others, the "protein-adsorption problem" on solid surfaces and incite studies on the underlying physico-chemical mechanisms of their interaction. Proteins represent an important class of biomolecules [1]. Whenever a material (device) is brought to a contact with a physiological fluid one of the first events to occur is adsorption of the freely available in the fluid proteins on the material surface [2–5]. The adsorption of a specific protein on the material surface is strongly affected by the presence of other proteins in the solution and by those already adsorbed on the material since according to the adopted conformation, the adsorbed proteins may expose new binding sites for subsequent protein–protein interactions. By adopting different conformations, the adsorbed proteins condition the surface, determine the biological response of the material and control further biological processes [6, 7]. Thus, in a bigger picture, the protein adsorption on solid surfaces represents the stage of conditioning of the surface, for example, for adhesion of microorganisms [8]. Microbial adhesion and subsequent biofilm formation are at the origin of hospital-acquired infections, often leading to septic complications and lethal issues [9]. This Public Health problem is at the core of actuality more than ever due to the emergence and selection of antibiotic-resistant bacteria which strongly limits the currently existing methods of treatment [10]. Accordingly, all related studies are timely actions. In line with this strategy we focus on the adsorption of proteins on solid surfaces. We investigate the interaction of proteins with solid surfaces of SiO_2 thin layers and with silver nanoparticles (AgNPs) deposited on the surface of SiO_2. Working with thin layers is related to creation of antimicrobial coatings. Silver, and particularly AgNPs, exhibits inherent antimicrobial properties. In the last decade, the antimicrobial activity of AgNPs has led to an extensive research of the involved molecular mechanisms, aiming at a broad application of silver-containing materials in the biomedical domain. It is well known now that the extreme biological activity of AgNPs is closely related to the released amount of ionic Ag (Ag^+) or to a direct contact of the microorganisms with AgNPs, resulting in protein denaturation at different cell locations [11–16]. The approaching to the surface microorganisms do not have specific receptors to bind the material. Actually, the already adsorbed on the surface proteins act as interface between the microorganisms and the material [8]. This complex and time-evolving system requires a close study of the interaction of proteins with silica surfaces and with silver species. The current contribution addresses adsorption, organization and conformational changes of two globular proteins, namely Bovine Serum Albumin (BSA) and Fibronectin (Fn) on thin silica layers without and with AgNPs, aiming at to assess their respective contributions.

II. EXPERIMENTAL PART

A. Substrate elaboration

The silica (SiO_2) layers were thermally grown on intrinsic Si-substrates at 1100°C under slightly oxidizing atmosphere, using a N_2–O_2 gas mixture with 1.0% of O_2. Before being exposed to proteins, the SiO_2/Si substrates were consecutively cleaned in ethanol (95% vol.) and acetone (95% vol.) and then rinsed in deionized water until obtaining zero conductivity to avoid electrostatic interactions on the sample surfaces.

The same SiO_2 thin layers were used to deposit a single plane of AgNPs on their surfaces. The AgNPs were obtained by Ag-sputtering for 5 s in an Ar-discharge (a radio-frequency capacitively-coupled plasma with an axial asymmetry of the electrodes) maintained at a pressure p_{Ar} = 5.4 Pa with a power P = 80 W (self-bias voltage of the powered electrode V_{dc} = – 950 V). More details on the plasma process and the deposition procedure can be found elsewhere [17, 18].

B. *Proteins*

Two globular proteins, namely BSA and Fn, were selected for this study. BSA has been chosen as a model protein and the choice of Fn was based on the large implication of this protein, as intermediate, in microbial adhesion. BSA and Fn were purchased from Sigma Aldrich. According to their SDS-PAGEs, the BSA was at least 96% pure and the Fn was at least 85% pure. Both proteins were in a lyophilized powder form.

Stock solutions of BSA and Fn were prepared with a concentration of 5.0 g/L in 50 ml water for injections (WFI water, European Pharmacopoeia, COOPER) with pH-value measured to 7.0 and conductivity of 1.2 µS/cm. Aliquots of the BSA and the Fn stock solutions were then diluted 2–100 times in WFI water. The measured pH-value of the stock solution was 5.6 for BSA and 7.5 for Fn. The assays were performed at room temperature (23°C).

The proteins were deposited on the silica surfaces and on the AgNPs by dip coating process [19]. The substrates were immersed for 1 hour in 1 mL of the protein solution to achieve protein adsorption. After the immersion step, the samples were rinsed in WFI water to remove all non-adhered proteins and were left dehydrating at room temperature and atmospheric pressure before characterization of the interaction of proteins with the selected surfaces.

C. *Characterization methods*

The SiO_2 thin layers, the AgNPs-patterned SiO_2 substrates and the adsorbed proteins were studied by Scanning Electron Microscopy (SEM), spectroscopic ellipsometry (SE) and Atomic Force Microscopy (AFM). A JEOL JSM 7800F Prime-EDS microscope was used to evaluate the obtained AgNPs and the adsorbed proteins. A Semilab ellipsometer SE-2000 with a rotating polarizer and a fixed analyzer was used in the spectral range 250–850 nm for the SE measurements. Bruggeman model was applied to extract the thickness and optical properties (refractive index, n and extinction coefficient, k) of the SiO_2 layers. Cauchy dispersion law was applied for interpretation of the recorded spectra of adsorbed proteins, since the two studied proteins are optically non-absorbing [19]. The protein surface concentration, *i.e.* the adsorbed protein mass per unit area, was obtained using the refractive indexes, after applying the de Feijter's expression [20]. AFM topography images were acquired with a Bruker Multimode 8 set-up using Peak-Force Quantitative NanoMechanical (PF-QNM) mode. To probe proteins a SNL tip with spring constant of 0.42 N/m and curvature radius of around 5 nm was used. The peak force was set to 0.5 nN.

III. Results and Discussion

A. *Structural analysis of the synthesized SiO_2 thin layers and AgNPs deposited on SiO_2*

The SE analysis of the thermally grown silica shows a high quality of the obtained thin SiO_2 layers. Their refractive index is n = 1.45, at λ = 632.8 nm. They are transparent. The extinction coefficient is k = 0 in the entire spectral range. The layer thickness is 84.4 nm. The same SiO_2 layers were used as substrates for the plasma deposition of AgNPs. SEM images (Figure 1(a)) of the AgNPs show that they are organized in a monolayer and are homogeneously distributed on the SiO_2 surface, covering 51% of it. Their shape can be defined as prolate spheroid. The AgNPs are positioned on the surface with random orientation of their major axis. They are well isolated from each other. As shown in Figure 1(b), the AgNPs are of size 19.7 ± 7.6 nm and follow a Gaussian distribution.

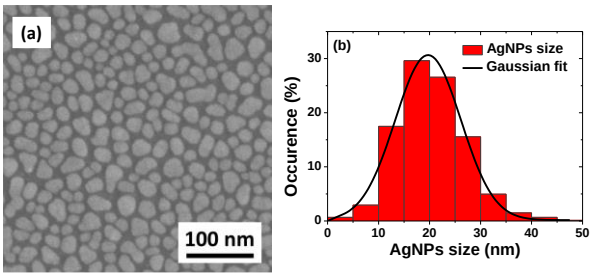

Fig. 1. (a) SEM image of the plasma synthesized AgNPs on SiO_2 thin layers and (b) statistical analysis made on more than 1000 AgNPs.

B. *Adsorption and organisational dynamic of BSA and Fn on the surface of SiO_2 thin layers*

The adsorption and organization of BSA (0.05 g/L in solution with WFI water) on the SiO_2 surface is presented in Figure 2, where Figure 2(a) shows the 3D-structure of BSA and indicates the protein size. The selected protein concentration is consistent with typical protein concentrations found in blood samples. Analysis of the SE spectra (Figure 2(b)) of the adsorbed proteins gives a protein monolayer with thickness of 4.1 ± 0.1 nm and refractive index of $n_{p\text{-}BSA}$ = 1.61. After applying the de Feijter expression one

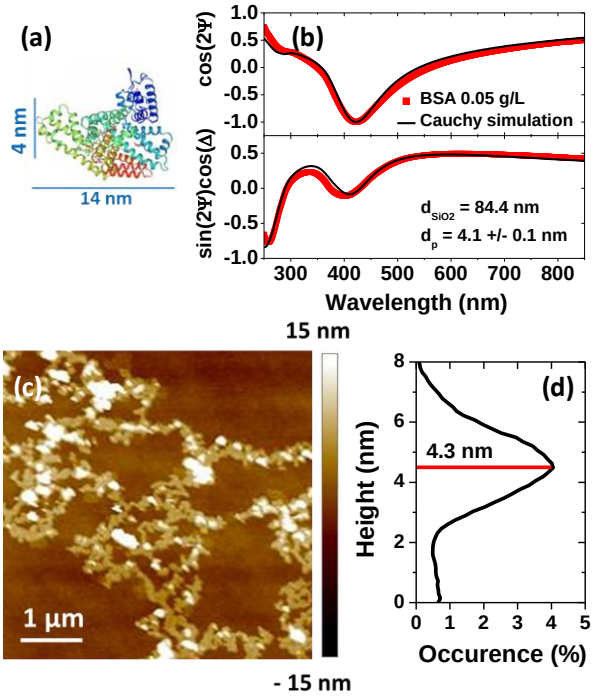

Fig. 2. Adsorption of BSA (from solution with concentration of 0.05 g/L) on SiO_2 surfaces: (a) representation of the 3D-structure of BSA, (b) Ellipsometric spectra of the adsorbed BSA, (c) PF-QNM AFM image of the protein organization and (d) height histogram of the adsorbed BSA.

finds a surface concentration of BSA $\Gamma_{BSA} = 0.58$ µg/cm^2. According to the AFM topography (Figure 2(c)) the adsorbed BSA is organized in a lace-like network on the surface. The height histogram resulting from PF-QNM analysis (Figure 2(d)) shows a single peak with value of 4.3 nm between the protein and the SiO_2 substrate. It means that for this small BSA concentration the resulting layer is non-continuous and the proteins are 'side-on' adsorbed on the surface. The obtained value is consistent with the BSA smaller dimension (minor axis of the spheroid, Figure 2(a)). It also perfectly correlates with the thickness of protein layer found from the SE analysis.

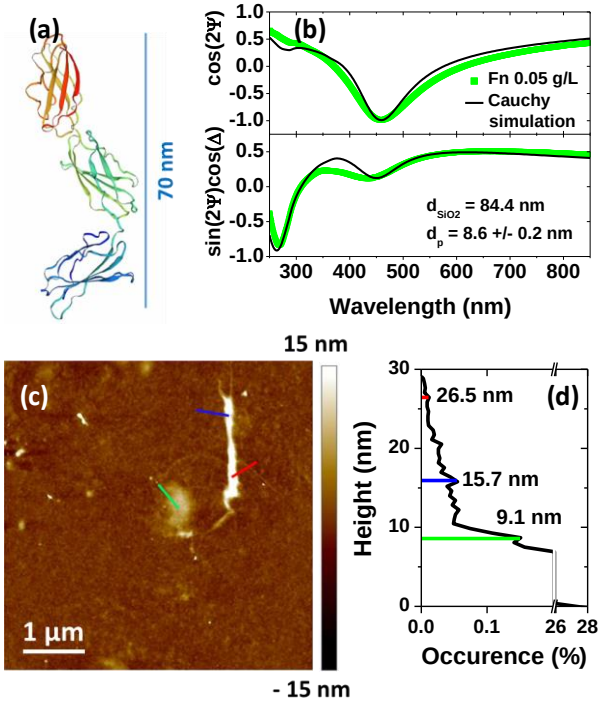

Fig. 3. Adsorption of Fn (from solution with concentration of 0.05 g/L) on SiO_2 surfaces: (a) representation of the 3D-structure of Fn, (b) Ellipsometric spectra of the adsorbed Fn, (c) PF-QNM AFM image of the protein organization, (d) height histogram of the adsorbed Fn.

Similar analysis was conducted for the resulting protein layers from Fn solution (0.05 g/L in solution with WFI water) (Figure 3). Fn proteins organize on the silica surface in a very different way compared to the BSA, due to structural differences and their much larger size (2 × 70 nm, Figure 3(a)). The obtained from SE protein layer thickness is of 8.6 ± 0.2 nm (Figure 3(b)). The extracted refractive index is $n_{p\text{-}Fn} = 1.62$, giving a surface concentration of $\Gamma_{Fn} = 1.32$ µg/cm^2. It is worth pointing out that one achieves twice larger surface concentration for Fn, compared to BSA, although starting with solutions with the same concentration.

The AFM topography study shows that in interaction with the SiO_2 surface, the Fn either fold up to achieve a globular shape or adopt more complex branch-type structures as shown in Figure 3(c). This is represented on the height histogram in Figure 3(d), where three characteristic heights can be observed. They are consistent with different Fn conformations. The most frequently occurring height is of 9.1 nm, which correlates well with the hydrodynamic radius of Fn in aqueous solution (8.7 nm [21]), and suggests globular compact conformation. The two other specific heights are of 15.7 nm and 26.5 nm. They can be assigned to partially unfolded and elongated protein conformations. It appears that the three conformational models for protein: globular (folded compact), branched, or linear (extended), can be assumed for the Fn after adsorption and dehydration on the silica surfaces.

The above analysis is confirmed by studies on adsorbed proteins from higher Fn concentrations. Figure 4 shows that the adsorbed proteins from solution with protein concentration of 1 g/L in WFI water organize in a fractal-type structure with nodes much higher that 300 nm and branches with average height of only 40 nm. It approves that other protein models, like complex random or looped ones also exist. These results validate the trend of Fn to adopt different conformations in interaction with SiO_2 solid surfaces.

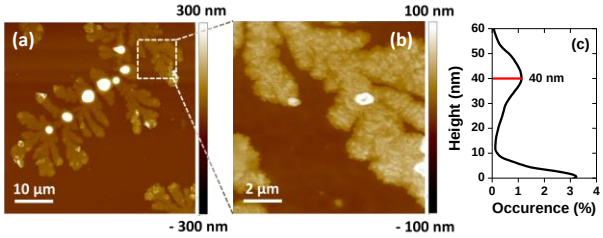

Fig. 4. PF-QNM AFM images of the organization of Fn (from 1 g/L in solution) on SiO_2 surfaces: (a) 50 × 50 µm and (b) zoom in the upper right part 5 × 5 µm and (c) the corresponding height histogram.

C. *Interaction of BSA and Fn with AgNPs-patterned SiO_2 surfaces*

The protein adsorption changes radically when the SiO_2 surface is patterned with AgNPs. On the other hand, in interaction with the AgNPs, the adsorbed proteins alter the release of the very reactive Ag^+ ion, by enhancing or depressing its amount [22]. Figure 5 shows the interaction of BSA (0.05 g/L in solution with WFI water) with the patterned by AgNPs SiO_2 surface. For this protein concentration one observes that BSA proteins organize in small islands encompassing the AgNPs in a region of around 100 nm in diameter. The islands are positioned at distances of at least 500 nm from each other (Figure 5(a)). A closer look (Figure 5(c)) suggests that the AgNPs are captured by the BSA proteins forming the so-called "protein corona". A consideration based only on electrostatic interaction cannot explain this globular compact conformation, since BSA (isoelectric point, pI = 4.7) and the surface of AgNPs both are negatively charged in the solution (pH = 5.6). Most likely the strong binding of BSA on the AgNPs is supported by sulfur-silver complexes, since BSA contains 583 amino acid residues and has 17 cysteine residues (8 disulfides bridges and 1 free thiol group) [23].

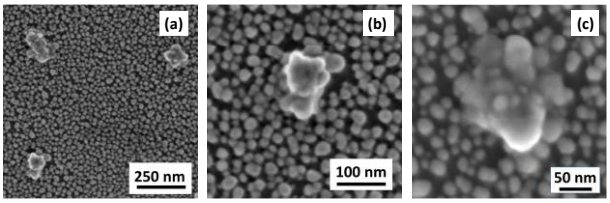

Fig. 5. SEM images showing deposition of BSA from a solution with concentration of 0.05 g/L on AgNPs.

The Fn has similar behaviour in interaction with the AgNPs (Figure 6). Again the globular compact conformation is observed, with islands positioned at distance of 500 nm. However, one can see (Figure 6(b)) that the Fn adopts also

unfolded and elongated protein conformations. Due to the much larger size of Fn, the region of interaction with the AgNPs is 3 times the protein size, *i.e.* around 400 nm in diameter (Figure 6(c)). Similarly, electrostatic interaction with the AgNPs is unlikely since the Fn (isoelectric point pI = 5.5 - 6.0) and the AgNPs are negatively charged in the solution (pH = 7.5). Again the thiol groups are in charge of the strong binding of Fn to the AgNPs. Although the SH-groups of free Fn are buried from the protein surface, they become fully exposed upon adsorption to a surface [24].

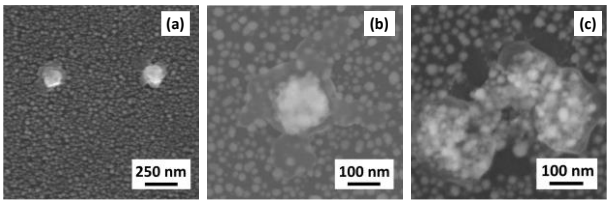

Fig. 6. SEM images showing deposition of Fn from a solution with concentration of 0.05 g/L on AgNPs.

Increasing the Fn protein concentration to 1 g/L does not seem to induce radical changes of the Fn adsorption on AgNPs-patterned SiO_2 layers (Figure 7). The globular compact type and the unfolded, and elongated protein conformations are preserved. However, the Fn organize mainly in a star-type shape, covering larger regions of AgNPs (3 – 5 µm).

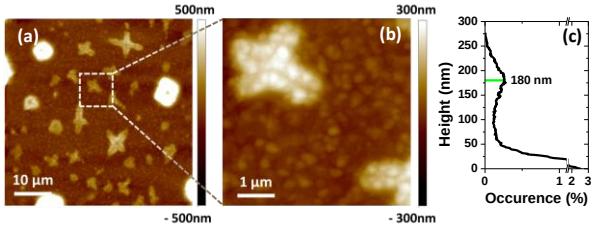

Fig. 7. PF-AFM images of the organization of Fn (from 1 g/L in solution) on AgNPs: (a) 50 × 50 µm and (b) zoom in the central part 5 × 5 µm and (c) the corresponding height histogram.

IV. Conclusions

Adsorption of BSA and Fn on SiO_2 surfaces leads to a lace-like and branching type organization, respectively. Although starting with the same protein concentration in solution, the amount of adsorbed on the SiO_2 surface proteins is systematically twice larger for Fn compared to BSA. Electrostatic interaction of both BSA and Fn with AgNPs is unlikely. The thiol groups are in charge of the strong binding of BSA and Fn to the AgNPs creating sulfur-silver complexes.

In the future we will study protein adsorption when the solution contains mixture of proteins. Such approach will answer questions related to protein-protein interactions and to protein dynamics driven by the Vroman effect. The protein adsorption on AgNPs surface is expected to be modified by the kinetic rates and by proper protein-AgNPs interaction.

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