Protein Binding to Gold Auto-assembled Films

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Abstract

Gold auto-assembled films were prepared from gold colloidal aqueous solutions on glass slides silanized with 3-aminopropyl-triethoxysilane at room temperature and with a thermal treatment at 200°C. The interaction of the major storage protein extracted and purified from aleurone cells of barley with the gold nanostructured films has been examined using absorption UV-Vis spectroscopy and atomic force microscopy (AFM). The UV-Vis spectra evidence the binding of major aleurone protein to gold nanostructured films. The AFM allowed the examination of the topography of the protein adsorbed films on gold auto-assembled films at the nanometric scale and confirmed that the protein forms a stable layer, following the morphology of gold nanostructured films decreasing the roughness of the gold surface. In all cases, protein was found to be adsorbed on gold surfaces and to form a stable protein layer.

Introduction

The deposition of gold nanoparticles on various surfaces with the formation of auto-assembled films was largely applied for different purposes, including analytical or biomedical applications (such as sensors), catalysts, physical studies (such as SERS effect), and for functionalization of the obtained gold layers [1-3]. As substrates a series of materials were used, such as silicon [4], SiO₂, glass, metals and metal oxides or carbon [4a]. The deposits of gold nanoparticles on bare glass supports present usually a low adherence, therefore a previous treatment of the glass surface is necessary. Usually organosilanes [5, 6] are used, based on the experience acquired in their application as fibre-matrix adhesion promoters in composites [7].

The greatest advance of the last decade in the study of nanoparticles and their aggregates, as well as their auto-assemblies in thin films on different solids, is the development of novel techniques to obtain *in situ* images of adsorbed aggregates, such as atomic force microscopy (AFM) [8-15] and transmission electron microscopy (TEM) [1, 15].

Previously, we reported the synthesis and characterization of three gold colloids with the average nanoparticle size from 10 to 50 nm using UV-Vis spectra and TEM observations [16]. Also, for the first time, we reported the results on the interactions of the major aleurone proteins with gold nanoparticles in colloidal aqueous solutions using UV-Vis spectroscopy and TEM observations [16]. The data indicated that aleurone protein binds to gold colloids.

In the present paper, we study the influence of gold nanoparticles auto-assembled as an interfacial film on the binding of major aleurone protein using UV-Vis spectroscopy. Also, the structure of nanostructured gold film coated with aleurone protein is analyzed using AFM observations. The findings suggest that aleurone protein is strongly bound with the gold film surface.

Experimental section

Materials

Gold auto-assembled films (also called nanostructured layers) were prepared from gold colloidal aqueous solutions, as described previously [16] by HAuCl₄ reduction with trisodium citrate. This gold colloidal solution had a gold content of 197 mg/L and an average particle size (diameter) of 24 nm, as determined from TEM images [16]. The gold films were obtained by placing a few drops of the said gold colloidal solution on glass slides, optically polished, freshly cleaned, initially silanized [17] with 3-aminopropyl-triethoxysilane. Then, by slowly drying of these gold nanostructured layers, the sample 1 was prepared at room temperature. Sample 2 was obtained by heat treatment of a sample 1 at 200°C in an oven for 2 hours. The 3-aminopropyl-triethoxysilane was purchased from Sigma and is of minimum 98% purity.

The protein used is the major aleurone protein, a globular

storage protein, extracted and purified from aleurone cells of barley (*Hordeum vulgare* L.) as shown elsewhere [18]. Solutions with a protein content of 2 mg/L were made with ultra pure water [16]. The pH of the protein solution was about 5.6.

The gold nanostructured layers coated by protein biomolecules were prepared by placing successively, at intervals of 3 days: 0.5; 1.0 and 1.0 mL (i.e., a total of 2.5 mL) of the 2 mg/L protein aqueous dispersion on about 1 cm² of the gold film surface of sample 1 and sample 2. Then, the gold films coated with protein were dried slowly at room temperature, and consequently, the films of nanostructured gold (sample 1 and sample 2) are coated by increasingly thicker adsorbed protein layers.

Methods

The UV/Vis absorption spectra of the colloidal gold and protein solutions and of the gold films were recorded using a Jasco UV/Vis V-530 spectrophotometer in the 190 – 900 nm wavelengths range.

Atomic force microscopy (AFM) investigations were made on the gold nanostructured films (samples 1 and 2) without and with adsorbed protein (samples 3 and 4), using a commercial AFM JEOL 4210 equipment with a 10 x 10 (x-y) µm scanner operating in tapping (noted also *ac*) mode. Standard cantilevers, non-contact conical shaped tips of silicon nitride coated with aluminum, were used. The tip was on a cantilever with a resonant frequency in the range of 200 - 300 kHz and with a spring constant of 17.5 N/m.

AFM observations were repeated on different areas from 10 x 10 µm² to 250 x 250 nm² of the same gold film. The images were obtained from at least ten macroscopically separated areas on each sample. All images were processed using the standard procedures for AFM. The sizes of nanoparticles were measured directly from AFM 2D-topographic images and their 3D-views. The thickness

(vertical distance) variations were estimated from vertical linear cross sections on AFM images, as shown elsewhere [8-15]. AFM images consist of multiple scans displaced laterally from each other in y direction with 512 x 512 pixels. Low pass filtering was performed to remove the statistical noise without to loose the features of the sample. All AFM experiments were carried out under ambient laboratory conditions (about 20°C) as previously reported [11-14].

Results and discussion

While the colloidal gold solution presented an absorption maximum at 529 nm [16], the aminopropyl silanized glass substrates coated with gold nanoparticles present an absorption maximum at 560 nm (sample 1, Figure 1a) and 543 nm (sample 2, Figure 1b). The shift of the surface plasmon band toward higher values of the wavelength can be attributed to the reduced nanoparticle to nanoparticle distance in the gold film compared with gold nanoparticles dispersed in water solution, in agreement with similar reported data [19].

The influence of adding the protein on gold nanostructured films is also pronounced. For sample 2 the maximum is shifted to 552 – 556 nm, depending on the quantity of protein added (Figure 1b), and for sample 1 the maximum shifts until 640 - 657 nm, with increasing amounts of protein (Figure 1a). The shoulder at lower wavelength in Figure 1a is due to the gold film in the slide areas where no protein solution was deposited. The new band at 640 nm is known to be characteristic for an electronic transition of aggregated Au particles [20].

As reported previously [16], the presence of the major aleurone protein on the gold nanoparticles deposited on TEM grids leads to the agglutination of the gold nanoparticles with

Figure 1

Optical spectra of nanostructured gold films on hydrophobic glass slides. (a) Sample 1 with protein; (b) Sample 2 with protein (solid line) and without protein (dashed line)

Figure 2

2D-topographic (a) and phase (b) AFM images of sample 1; scanned area 500 x 500 nm²; (c) 3-D view of the same area. (d) Cross section profile along the arrow in Fig. 2a

the development of large aggregates. A similar effect could explain the modified spectra for gold nanoparticles deposited in thin films, sample 1 (Figure 1a) and sample 2 (Figure 1b).

Further, the results of AFM investigations on the nanostructured gold films, before and after adding the protein solution, are presented in Figures 2-7. One of the advantages of AFM over TEM is that AFM can offer the possibility to determine the distance along the z-axis perpendicular to the plane of the film surface. Since the surface of the gold film is quite ordered, the x-axis can be defined as any line connecting the adjacent particles as shown in Figures 2-7, panels a. Plots of the film surface

contour can be obtained by various cross sections through the film in the x-z plane (see, Figures 2 and 3, panel d, and Figures 4-7, panel c). The film surface in all these images has a rather ordered structure, which might be roughly related with the size of the film nanoparticles [8,10,15a]. The film surface roughness is measured by the root mean square, RMS, value given in Table 1. The Δz value is obtained from peak to valley distances in the profiles and is given also in Table 1.

Figure 2 displays the top view of the nascent gold nanostructured film (sample 1) prepared by self-assembling of gold nanoparticles on silanized glass at room temperature, while Figure 3 depicts the gold film heated for two hours at

Table 1

The film surface roughness, given by the root mean square, RMS, values (Figures 2a-7a) and Δz values, obtained from peak to valley distances in the profiles (Figures 2d and 3d, and Figures 4c-7c), for the gold nanostructured films in the absence and in the presence of protein, for scanned areas of $0.500 \times 0.500 \mu\text{m}^2$ and $0.250 \times 0.250 \mu\text{m}^2$

Gold film	RMS, nm	Δz , nm
Sample 1	3.5	9 – 12
Sample 1 with 0.5 ml protein (2 mg/L) aqueous solution	2.9	7 – 10
Sample 1 with 2.5 ml protein (2 mg/L) aqueous solution	1.8	3 – 6
Sample 2	6.8	18 – 24
Sample 2 with 0.5 ml protein (2 mg/L) aqueous solution	4.8	12 – 16
Sample 2 with 2.5 ml protein (2 mg/L) aqueous solution	1.8	4 – 7

200°C (sample 2). From these figures, it is clearly observed that the gold nanoparticles are aggregated and oriented in rows in nascent gold film (Figure 2, 2D-topographic (a) and phase (b) images, 3D-view (c)), but are uniformly distributed and packed in distorted pentagons or hexagons in the thermally treated film (Figures 3a, b and c). The cross section of the gold film surface shows that there is a quite sharp junction between the gold rows (Figure 2d) in the nascent film and between neighboring gold nanoparticles in the gold film heated at high temperature (Figure 3d). This could explain the shift of the UV-Vis absorption peak towards longer wavelength in nanostructured gold films (Figures 1a and 1b) as compared to gold colloidal solutions [16], since particle aggregation has this effect [21]. The cause resides in electric dipole-dipole interaction and the coupling between plasmons of neighboring particles inside the aggregates, when the interparticle distance is smaller than the particle diameter [22], as found in our cases (Figures 2 and 3).

Taking into account the data about morphology and cross sections of the nanostructured gold films, which were repeated over six months, it would appear that the films made either at room temperature (sample 1) or aged at high temperatures (sample 2) have a different but rather stable surface structure. For experimental reasons it is not possible to have exactly the same scanned areas in films after each heating or protein adsorption step. Thus, the images in Figures 2 to 7 refer to slightly different regions of the gold film surfaces in the absence or presence of protein. Comparisons among these different regions are nevertheless meaningful, since a great number of film regions were scanned and the images demonstrated the film surface uniformity for every independent preparation.

Before the nascent gold film is completely dry, it is reasonable to presume that the gold particles order, come into contact and aggregate on silanized glass support. It

appears that a deformation process accompanies the film drying process and the aggregated gold particles form ordered rows with quite sharp junctions between them within the gold film (sample 1, Figure 2).

Upon heating of the nascent gold film (sample 1) at high temperature, there is a rearrangement of gold particles and particle boundaries which is observed in the resulting sample 2, which is slowly cooled from high temperature to the room temperature. The structure of the gold film surface of sample 2 shows non aggregated gold particles, rather well ordered into distorted pentagons and hexagons (sample 2, Figure 3). The increase of Δz values (Table 1) might be considered as a manifestation of an effective increase in gold nanoparticles by diffusion.

Figures 4 and 5 display the nascent gold film (sample 1) coated with the two different quantities of protein, while the Figures 6 and 7 are those of a gold film (sample 2) which was prior heated at high temperature, under approximately the same coating protein layers as for Figures 4 and 5, respectively. The size and shape of gold nanoparticles on the gold film surface is clearly preserved in the presence of protein molecules that are adsorbed and probably form cross-links among gold nanoparticles increasing the gold films stability, for either the nascent film or the heated film at high temperature.

The loss of contrast in the AFM images of the samples 1 and 2 with high protein concentrations in adsorbed layers (Figures 5 and 7), corresponds to a diminishing effect of the film surface roughness (see RMS values given in Table 1) and probably to a fading of boundaries among adjacent gold nanoparticles. The vertical dimensions, Δz , (Table 1) are much smaller (Figures 4c and 6c) or present only mild undulations (Figures 5c and 7c) for gold films coated with protein than in the nascent film (sample 1, Figure 2d) and particularly than in the heated film at 200°C (sample 2, Figure 3d). The surface structure of these gold films coated with thicker protein adsorbed layers has almost disappeared, especially for the heated gold film (Figure 7).

Occasionally, a few rounded shapes can be observed (Figure 5a and Figure 7a) and they probably correspond to initial gold nanoparticles coated by protein or to new aggregates formed by adsorbed protein molecules. This finding supports the idea that the spaces between gold nanoparticles are filled up by protein during the protein adsorption process. The protein molecules are presumably strongly adsorbed on nanostructured gold films and this process would seem to lead to a decrease of the diffusion of gold from neighboring gold particles and, therefore, they stabilize the surface of the gold film.

On the other hand, the images in Figures 4 and 6, corresponding to the lower protein concentration, show a high degree of order similar with that found in pure gold films without protein, as shown in Figure 2 (sample 1, gold nanoparticles oriented in rows) and Figure 3 (sample 2, gold nanoparticles packed in distorted pentagons or hexagons), respectively. For example, see the marked area in Figure 4A (a), rescanned in Figure 4B (a), and the marked area in Figure

Figure 3

2D-topographic (a) and phase (b) AFM images of sample 2; scanned area 500 x 500 nm²; (c) 3-D view of the same area. (d) Cross section profile along the arrow in Fig. 3a

6A (a), which is rescanned in Figure 6B (a). This indicates that the protein ultrathin adsorbed layers, at low protein concentrations, follow the shape of gold nanoparticles of the gold film surface of either sample 1 or sample 2. This finding also suggests that protein conformational changes might occur by its spreading on the gold surface.

These observations are consistent with the shifts in UV-Vis spectra after adding protein, as an effect of the particles aggregation, in substantial agreement with reported data [16, 23, 24]. Thus, studies on the influence of oligonucleotides on the optical properties of gold nanoparticles also showed that the most important parameter in controlling the spectral

shift is the aggregate size [23].

A similar effect was observed for the adsorption of 1,4-phenylenediisocyanide on different sized gold nanoparticles, resulting in an aggregation of these particles [24]. For small particles, there was only a slight red shift in the UV-Vis absorption spectrum, indicating a minor decrease in interparticle distance, while for increased size of the particles the shift was important.

The secondary structure of the major aleurone protein was recently investigated by FTIR [25], and FT-Raman and NMR [26] spectroscopy. The data indicate α -helix, β -sheet, β -turns and random coil structures.

Figure 4A

2D-topographic (a) and 3D- (b) AFM images of sample 1 with a low quantity of protein adsorbed on the gold film; scanned area 500 x 500 nm²; (c) Film surface contour along the arrow in panel a

Figure 4B

2D-topographic (a) and 3D- (b) AFM images of the marked area (250 x 250 nm²) in Fig. 4A a. (c) Film surface contour along the arrow in panel 4B a

Figure 5

2D-topographic (a) and 3D- (b) AFM images of sample 1 with a high concentration in adsorbed protein; scanned area 500 x 500 nm²; (c) Film surface contour along the arrow in panel a

Figure 6A

2D-topographic (a) and 3D- (b) AFM images of sample 2 with a low quantity of protein adsorbed on the gold film; scanned area 500 x 500 nm²; (c) Film surface contour along the arrow in panel a

Figure 6B

2D-topographic (a) and 3D- (b) AFM images of the marked area (250 x 250 nm²) in Fig. 6A a. (c) Film surface contours along the arrows in panel 6B a

Figure 7

2D-topographic (a) and 3D- (b) AFM images of sample 2 with a high concentration in adsorbed protein; scanned area 500 x 500 nm²; (c) Film surface contour along the arrow in panel a

The composition in amino acids of major aleurone protein, namely the N-terminal sequence [18] of the 16 amino acids is the following: ¹Glu ²Gln ³Gly ⁴Asp ⁵Ser ⁶**Arg** ⁷**Arg** ⁸Pro ⁹Tyr ¹⁰Val ¹¹Phe ¹²Gly ¹³Pro ¹⁴**Arg** ¹⁵(Ser or His) ¹⁶Phe. It is to be noted that arginine residue is repeated three times in the N-terminal of aleurone protein. Also, the arginine content of 7.9% and a lysine content of 5.2% were determined in the aleurone protein composition [18].

Both lysine and arginine residues of the aleurone protein carry their side amine groups positively charged in the working conditions [18], which give a polycationic character to the protein. These groups are capable to form salt bridges with carboxylate from citrate adsorbed on gold nanoparticles [16]. Citrates were found to be stable until temperatures over 200°C [27], so it should be present also in the heated film. Further, the protein binding to the gold film could lead to its stabilization by forming cross links among gold nanoparticles, for instance via electrostatic interactions between negatively ionized groups (-COO⁻) from aspartic and glutamic acid, both present in the protein, and positive groups on a protein molecule bound to another gold nanoparticle.

From this supposed role of strong electrostatic binding it does not necessarily follow that other contributions are excluded, such as those from steric or hydrophobic interactions. Also, due to the adsorption of protein molecules on the nanostructured gold film, structural modifications leading to the unfolding (denaturation) of aleurone protein molecules within the protein film might appear during the adsorption and the drying periods.

These findings are in agreement with the data obtained on the bovine serum albumin (BSA) binding to citrate coated gold surfaces, using quartz crystal microbalance and ζ-potential measurements [28], which showed that the binding occurs by a dominant electrostatic mechanism.

Conclusions

UV-Vis spectra and atomic force microscopy (AFM) observations on nanostructured gold films contribute to the knowledge and understanding of the structure of gold nanoparticle films and of their interaction with the protein from aleurone cells of barley.

From our experimental data it can be inferred that the aleurone protein is bound to gold nanostructured films as it was to gold nanoparticles in colloidal aqueous solutions [16], but the interaction is stronger with the auto-assembled gold films. The binding of protein to citrate coated gold nanoparticles should occur primarily by electrostatic interactions, such as salt-bridges between the citrate and the lysine or arginine positively charged on the protein surface. However, this does not exclude contributions from steric or hydrophobic interactions or structural modifications within the protein film during the adsorption and drying periods.

The AFM studies reveal the differences between the native nanostructured gold film and the same film after a heat treatment. The AFM investigations give the topography of the protein adsorbed on gold at the nanometric scale and confirm that the protein forms a stable adsorbed layer, following the morphology of the nanostructured gold film, and decreases the roughness of the gold surface. These data suggest that the protein conformational changes (e.g., unfolding) might occur by protein spreading on the gold film surface.

In all cases, protein was found to be irreversibly adsorbed on gold and to form a stable protein layer. These findings also suggest the protein film on gold to be well suitable as a biological multifunctional layer for industrial and biomedical applications.

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