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Self-assembly characteristics of gold nanoparticles in the presence of arginine

L. BARBU-TUDORAN, GH. TOMOAIA[a], O. HOROVITZ[b], A. MOCANU[b], M. TOMOAIA-COTISEL[b*]
Babes-Bolyai University, Electron Microscopy Center, 400006 Cluj-Napoca, Romania
[a]*Iuliu Hatieganu University of Medicine and Pharmacy, Orthopedic Surgery Department, 400015 Cluj-Napoca, Romania*
[b]*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 400028 Cluj-Napoca, Romania*

The interaction of colloidal gold in aqueous solutions with L-arginine solutions of different concentrations and in various mixing ratios was investigated by UV-Vis spectroscopy, transmission electron microscopy (TEM) and atomic force microscopy (AFM). It is evidenced that L-arginine mediates the formation of assemblies of gold nanoparticles, which are shown in TEM and AFM observations to have a complex structure. The TEM results for the evolution of these assemblies are used to evidence the progress in the building process of supermolecular structures. A model is proposed for the mediating action of arginine in the interparticle interactions.

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Keywords: Gold nanoparticles, Self-assembly, L-Arginine

1. Introduction

The functionalization of gold nanoparticles by amino acids is an important research subject from the point of view of forming bioconjugates of proteins or DNA with gold nanoparticles [1-3]. Amino acids, such as L-arginine, are suitable organic molecules to initiate the self assembly of gold nanoparticles.

In particular, L-arginine appears to be a good organic molecule model (being positively charged in a large interval of pH) to study the self assembly characteristics of gold nanoparticles negatively charged in aqueous solutions. L-arginine prefers to be on the outside of protein chains [4] and, therefore, it can be used in order to understand the interactions between proteins and gold nanoparticles [5,6].

L-Arginine contains a guanidinium group, -NH-$C(NH_2)_2^+$, besides the usual amine and acid groups in the molecule (Fig.1). From its pK-values: 2.17, 9.04 and 12.48 [7], it results that the molecule is predominantly positive (protonated) at physiological pH and therefore hydrophilic.

The complex guanidinium terminal group of L-arginine chain has a geometry and a charge distribution that is ideal for binding negatively charged groups, so it should preferentially bound to negatively charged gold nanoparticles.

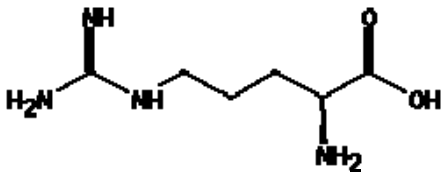


Fig.1. *L-Arginine formula*

Gold-silver nanocomposites were prepared from gold nanorod seeds in arginine solutions [8]. The use of L-arginine as reduction and capping agent for silver or gold nanoparticles was discussed [9, 10] but the self assembly mechanism is not completely understood.

Previously Maniu et al. [11] have studied the interaction of p-aminothiophenol with gold nanoparticles

In the present study, the interaction of L-arginine with gold nanoparticles was investigated, having in view the potential effects of amino acids in the formation of protection layers for the metal nanoparticles, in the mediation of nanoparticles self assembly, and even as reduction agents for the preparation of gold nanoparticles [3].

2. Experimental

The colloidal gold solution was prepared by $HAuCl_4$ reduction with sodium citrate, by a variant of the Turkevich method [12,13], as adapted from [14]. 200 mL 0.005% (w/w) $HAuCl_4 \cdot 3H_2O$ solution stirred vigorously was refluxed. To the boiling solution 15.3 mg trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), solved in a minimum amount of water, was added. After color change, the heat was turned off and the solution was allowed to cool overnight to room temperature. The resulting solution of colloidal gold particles was stored in a brown bottle and kept at 4 ^oC. The gold content in the final sol is 25 mg/L.

The tetrachloroauric (III) acid was purchased from Merck (high purity above 99.5 %). The trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) was obtained from Sigma Aldrich (high purity above 99%). Deionized water with resistivity of 18 MΩ·cm was used in all experiments and it was obtained from an Elgastat water purification system.

Solid L-arginine was purchased from Sigma and used without further purification; it was dissolved in deionized water (pH 5.6) in order to prepare 0.01and 0.1 M solutions, having a working pH value of 6.

The UV-Vis absorption spectrum of the aqueous solutions was recorded using a Jasco UV-Vis V-530 spectrophotometer with 10 mm path length quartz cuvettes in the 190 – 900 nm wavelengths range.

The investigated mixtures were obtained from the gold colloidal solution (c_{Au}) and the arginine solutions (concentration c_{Arg}), by successive removal of small amounts of the previous mixture and adding of equal amounts of amino acid solution. The gold to arginine content ratios reported to (c_{Au}/c_{Arg}) are given in the figures representing their spectra.

The gold nanoparticles suspension in the absence and in the presence of arginine was deposited and air dried on the specimen grid and observed with a transmission electron microscope (TEM: JEOL – JEM 1010). TEM specimens consist of carbon or collodion coated copper grids. TEM images have been recorded with a JEOL standard software.

Atomic force microscopy (AFM) investigations were executed on the gold nanostructured films made from gold nanoparticles functionalized with arginine, using a commercial AFM JEOL 4210 equipment operating in tapping (noted *ac*) mode [15] on thin adsorbed gold films on glass plates, that are optically polished and silanized with 3-aminopropyl-trietoxysilane. Standard cantilevers, non-contact conical shaped of silicon nitride coated with aluminium, 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 μm^2 to 250 x 250 nm^2 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. AFM images consist of multiple scans displaced laterally from each other in y direction with 512 × 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 ^oC) as previously reported [16].

3. Results and discussion

The aqueous colloidal gold solution was characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM). The visible absorption spectra of the gold sol (Fig. 2) presents a well-defined absorption band with a maximum at the wavelength λ_{max} = 527 – 528 nm [8], characteristic for plasmon absorbance in nanometric gold particles.

The size of the gold colloid particles has been measured by TEM imaging. The particles show mostly spherical or elliptical shape. From the sizes of a great number of particles, measured on the TEM images, the characteristics of the gold sample were calculated: average size (diameter): 14.2 nm, with a standard deviation of 2.6 nm; average mass of a particle (considered spherical): 2.9×10^{-17} g; average number of gold atoms in a particle 8.8×10^4 and number of gold particles per cm^3 of solution: 8.6×10^{11}. The colloidal gold solution proved as very stable in time, without observable modifications in the UV-Vis spectrum a year after preparation. This indicates electrostatic stabilization via gold nanoparticle surface-bound citrate anions.

The ***UV-Vis spectrum*** of L-arginine presents no absorption bands in the range of wavelengths investigated here. The absorption maximum for L-arginine lies in UV, at 194 nm.

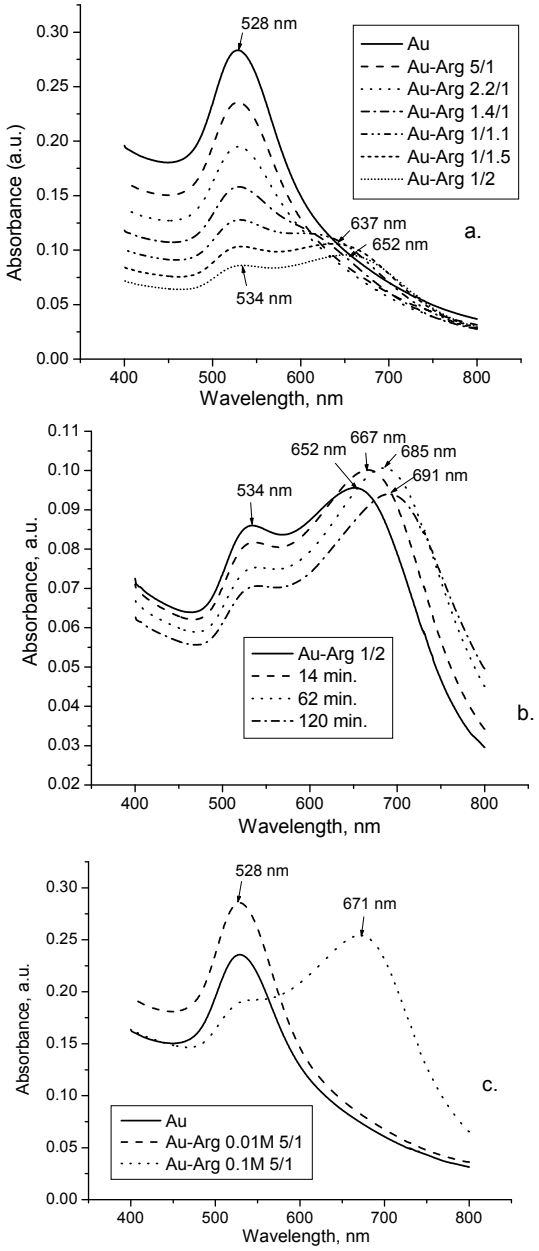


Fig .2. Optical spectrum of colloidal gold solution with 0.01 M arginine solution in different ratios (a); its time evolution (b), and with arginine solutions of different concentrations (c).

For small amounts of the diluted arginine solution (0.01 M) added to the colloidal gold solution (Fig. 2a), the spectrum is only slightly modified. Besides the lowering of the absorbance due to the dilution of the gold solution, the absorption maxima are lightly shifted towards longer wavelength. This shift is due to the change in the dielectric constant in the adsorption layer, the increase of the average refractive index of the environment surrounding the nanoparticles, and to the size increase of particles by the adsorbed layer [12].

However, for the 1.4:1 (c_{Au}/c_{Arg}) gold / L-arginine ratio, a second absorption band appears at higher wavelengths, partially overlapped with the initial absorption band. By further adding L-arginine, the new band broadens and its maximum shifts to 640-650 nm (Fig.2a). This band, characteristic for the aggregation of nanoparticles, is due to the coupling of surface plasmon resonance of neighboring nano-particles and is an indication of the anisotropic optical properties of the gold nanoparticles aggregates [17].

The time evolution of the self-assembly process is shown in Fig. 2b for an Au-Arg (0.01 M) ½ ratio. The maximum for aggregates is shifted further towards longer wavelengths, while the original maximum for individual gold nanoparticles (at about 530 nm) is decreasing and eventually reduced to a shoulder. As a consequence of the bathochromic shift of the absorption band the color of the solution changes from reddish to blue. The adding of a more concentrated (0.1 M) L-arginine solution leads directly to the formation of the aggregates and to the color change of the solution (Fig. 2c).

TEM images for gold nanoparticles with a 0.01 M L-arginine solution (Fig. 3) show complex assemblies of nanoparticles, linked together by means of amino acid molecules. The progress of aggregation in time for the mixture can be followed in this figure, by images taken at 1, 30 and 60 min. after mixing the colloidal gold solution with 0.01 M L-arginine solution in the 1:1 volume ratio.

AFM images for assembled gold nanoparticles with L-arginine are shown in Fig. 4. The AFM images were acquired with high resolution despite the structural complexity of molecules or of gold nanoparticles arrangements [6, 12, 13].

The assembly of gold nanoparticles, mediated by L-arginine was deposited on planar hydrophobic glass or on positively charged glass surfaces. Then, the assembly was observed by AFM under tapping mode and the structural features are visualized in Fig. 4. The two-dimensional topographic image for L-arginine mediated gold assemblies (Fig. 4a) shows the morphology of an almost ordered structure within domains, with small defects at domain boundaries. For this assembly of gold nanoparticles mediated by L-arginine, immobilized on planar glass surface, individual gold nanoparticles are visible from 2D-topography (Fig. 4a) and 3D-view (Fig. 4b). The gold nanoparticles appear almost ordered both in AFM images and in cross section profile (Fig. 4c). Also, AFM images evidence both the selectivity of L-arginine adsorption and its orientation on gold surface leading to aggregates as observed by UV-Vis spectroscopy.

Therefore, L-arginine is one of the amino acids found to interact strongly with the gold nanoparticles and to initiate their aggregation. L-arginine falls in the class of "polar" amino acids, defined as those with side groups that prefer to reside in an aqueous environment. For this reason, one generally finds these amino acids exposed on the surface of a protein [4]. Further, all the "charged" amino acids present such strong interactions, e.g. both the amino acids that are *positive* (protonated) at physiological pH, such as arginine and lysine, and those that are *negative* (i.e. de-protonated) at physiological pH, like glutamic acid and aspartic acid [1]. All these amino acids, after adsorption on the gold particles, have still two functions free to form bonds between particles.

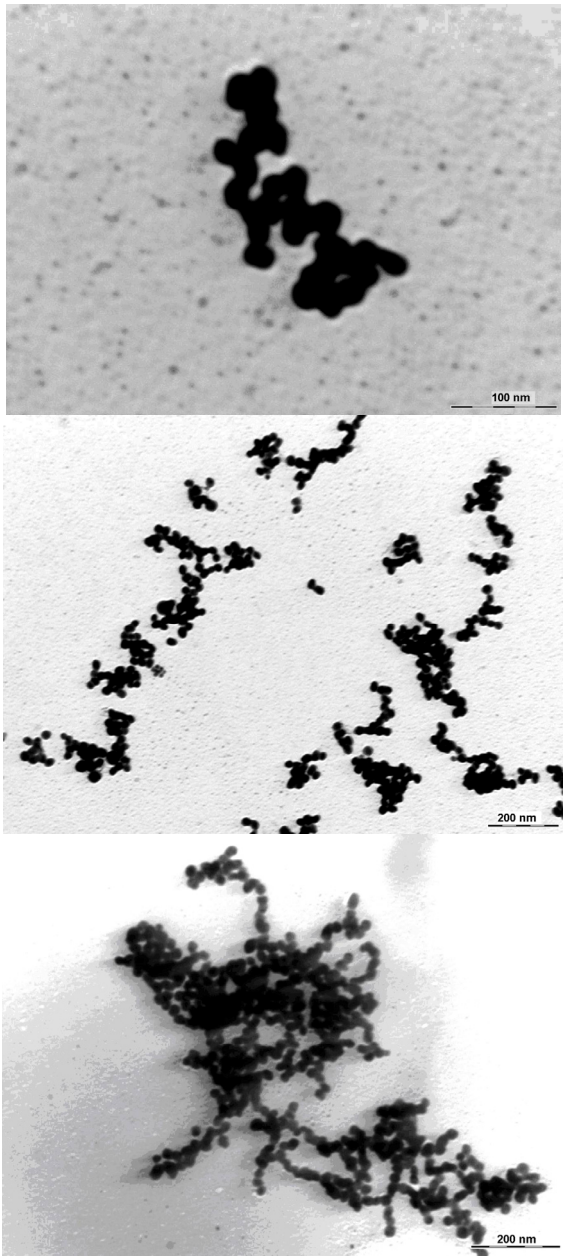
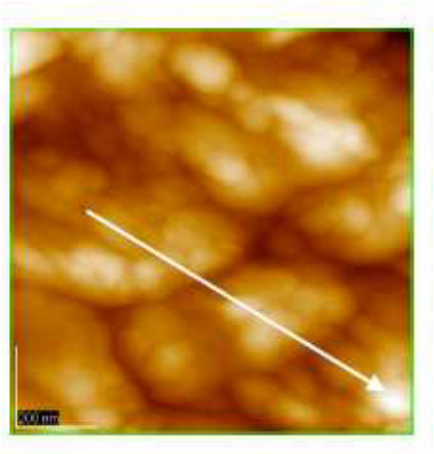
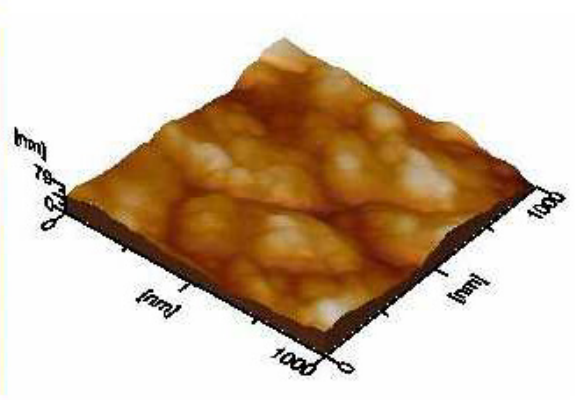


Fig. 3. TEM images of gold nanoparticles capped with L-arginine (0.01 M),1:1 vol. ratio, at different times after preparation: (a) 1 min; scale 100 nm; (b) 30 min., scale 200 nm; (c) 60 min; scale 200nm.



a



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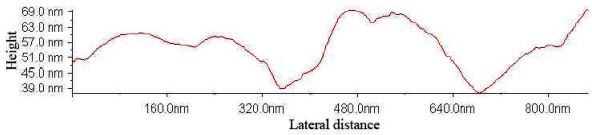

c

Fig. 4. 2-D topographic AFM image (a) of the L-arginine mediated assembly of gold nanoparticles, deposited on hydrophobic glass; scanned area 1000 × 1000 nm^2; 3D-view (b) of the topography (a); (c) cross section profile along the arrow in panel a.

A possible L-arginine binding to gold nanoparticles and the formation of particle assemblies and aggregates is schematized in Fig. 5. The L-arginine molecule, in its stable form for usual pH values of physiological medium or as in our working pH solutions has its positively ionized guanidinium group anchored on the negatively charged gold nanoparticles. By means of the free $–NH_3^+$ and $–COO^-$ groups of L-arginine, electrostatic bonds are possible between amino acid molecules bound to different gold nanoparticles, thus linking these particles. Of course, other linkage possibilities, such as hydrogen bonds, can also be considered.

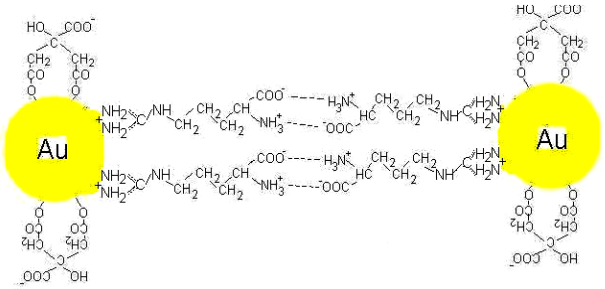

Fig. 5. Schema of L-arginine binding to citrate capped gold nanoparticles and links formation between gold nanoparticles

4. Conclusion

The UV-Vis, TEM and AFM investigations on citrate capped gold nanoparticles with aqueous L-arginine solutions converge in the evidencing the existence of strong interactions between the gold particles and the amino acid and the mediating action of L-arginine in the self assembly of gold nanoparticles. We suggest that the positively charged guanidinium group of L-arginine is anchored on the negative citrate capped gold nanoparticles reducing the energy barrier of L-arginine adsorption on gold surface and subsequently, L-arginine-capped gold nanoparticles facilitate the surface bonding and the interparticle interactions. The other two functionalities of L-arginine are involved in the bonding between L-arginine monolayers adsorbed on gold nanoparticles. As L-arginine is usually present in significant quantities in protein structure [17], we can infer on the role of L-arginine in the interaction of proteins with gold nanoparticles, in substantial agreement with our earlier findings [5, 6].

Thus, L-arginine offers a good approach for building molecularly defined surfaces with controlled surface characteristics (e.g., chemical composition and surface charges) relevant to wide applications ranging from micro- and nanofabrication to biological and medical sensing.

Acknowledgement

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*Coresponding author: mcotisel@yahoo.com