Electrochemical Preparation and Delivery of Melanin–Iron Covered Gold Nanoparticles

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Thiol-capped gold nanoparticles modified with iron-melanin are attractive because they combine magnetic properties and biocompatibility. The biopolymer modified nanoparticles are prepared and delivered following a three step strategy: i) adsorption of thiol-capped metal nanoparticles on graphite, ii) electrochemical deposition of melanin-iron, iii) potential-induced delivery of the modified nanoparticles to the electrolyte.

Introduction

The preparation of metallic nanoparticles (NPs) modified with organic, bioorganic or oxide coatings is appealing because of their wide range of potential biological and technological applications in the emerging fields of nanoscience and nanotechnology.[1] In this context, the development of new strategies capable of functionalizing NPs with complex molecular systems by using simple and inexpensive methods is a frontier topic that deserves special attention.

We have recently demonstrated that thiol-capped AuNPs can be adsorbed on highly oriented pyrolytic graphite (HOPG) by simple immersion in AuNP containing solutions,[2] and that the amount of immobilized thiol-capped AuNPs depends both on their concentration in solution and on the adsorption time. Efficient release of most of the adsorbed nanoparticles takes place during thiol electrodesorption, allowing the delivery of the AuNPs to a specific environment by applying small potentials.[2] On the other hand, bioorganic-iron coatings are interesting because they confer biological compatibility to metallic nanoparticles, while iron could be used to guide them to specific targets by applying a magnetic field. Melanins are complex biopolymers that are widely distributed in living organisms.[3] Eumelanins formed by polymerization of 5,6-dihydroxindole (DHI) and 5,6-dihydroxindole carboxylic acid (DHICA) monomers are the most abundant class of melanins. Eumelanin, in particular, possesses interesting physicochemical properties:[3] a strong, broad UV-band and visible absorption, extremely low radiative quantum yield,[4] anti-oxidant and free radical scavenging ability,[5] and electrical conductivity and photoconductivity in the condensed phase.[3] These biopolymers are able to coordinate a large number of metallic ions.[6] The affinity of melanins for metal cations has been studied in relation to melanome cell targeting by different drugs.[7] Recently we have shown that melanin-iron films can be prepared by electrodeposition on Au surfaces in a controlled way.[8]

Herein, electrochemistry is used to modify nonanethiol (NT) capped AuNPs supported on HOPG with melanin iron and then to deliver the modified NP to a preset environment by re-ductive electrodesorption. The melanin–iron modified NT–AuNPs are characterized by X-ray photoelectron spectroscopy (XPS), high resolution transmission electron microscope (HRTEM), small angle X-ray scattering (SAXS) and electrochemistry.

Results and Discussion

NT–AuNPs were immobilized on HOPG substrates by immersion in AuNP hexane solutions for 24 h following the procedure reported in ref. [2]. Melanin–iron films were then deposited on the NT–AuNPs by polarization of the HOPG substrate in a melanin containing 0.1 M NaOH solution for 2 hs at −1.0 V, as reported in ref. [8]. After melanin–iron deposition and careful rinsing, XPS spectra of the samples were recorded (Figure 1, black dots). The curves in the Au 4f (S 2p) regions have two components split by 3.65 eV (1.19 eV) with a branching ratio of 0.75 (0.5) that accounts for the spin-orbit coupling. The Au $4f_{7/2}$ component at BE=84.0 eV indicates that gold is metallic (Figure 1a), while the S $2p_{3/2}$ component at BE=162.0 eV (Figure 1b) has been ascribed to bounded thiols.[9–12] From these

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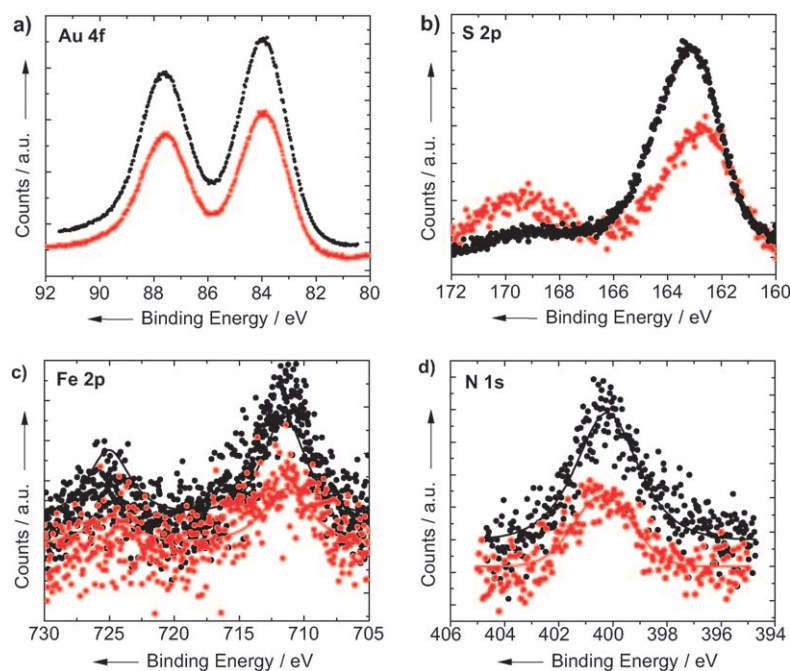Universidad de la Laguna, Tenerife (Spain)

Figure 1. XPS data for NT–AuNPs on HOPG after melanin–iron deposition (black dots) and for NT–AuNPs adsorbed on HOPG from hexane solution after extraction of modified NT–AuNPs from the 0.1 M NaOH solution and readsorption (red dots). a) Au 4f, b) S 2p, c) Fe 2p and d) N 1s. The lines in Figures (c) and (d) are used as a guide to the eye.

results the presence of thiolate capped-AuNPs on the HOPG surface can be confirmed.[2]

Moreover, since the S 2p spectra can be fitted without any additional S $2p_{3/2}$ components at BE = 163–164 eV, we can conclude that there are no unbounded thiols in our samples .[2] More important, we also observe the typical signals corresponding to melanin–iron: oxidized Fe (Figure 1 c) and N (Figure 1 d).[13] The presence of melanin–iron on the AuNPs was then confirmed by voltammetric runs. In Figure 2 we show the response of the NT–AuNP–HOPG sample after melanin–iron modification. The voltammetric profile is similar to that reported for the melanin–iron system on Au(111),[8] with a broad peak that involves contributions from both quinone/hydroquinone and iron oxides redox couples.

Concerning the XPS spectra shown in Figure 1 we don't observe important changes in the S/Au signal ratio before and after melanin–iron modification, indicating that no significant
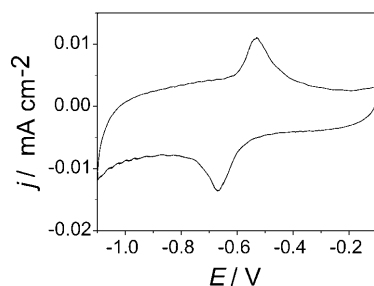
amount of NT is desorbed during melanin deposition at −1.0 V. In fact, Figure 3 shows that the reductive desorption peak of NT from the AuNPs is located at −1.3 V, that is, at potentials more negative than those observed in planar Au surfaces.[14]

However, it is evident that defects in the adsorbed thiol layer can be induced during the prolonged polarization at −1.0 V, where a very small cathodic current starts to be observed. These defects are preferred regions where melanin–iron can be deposited. On the other hand, the XPS data in Figure 1 also show that the Fe/N signal ratio is ≈0.15, that is, smaller than the 0.25 expected for a complete saturation of melanin protomolecules.[8]

XPS and voltammetric data for clean HOPG samples kept in contact with the melanin-containing solution at the same potential and for the same time show no significant amounts of melanin–iron. Therefore, we can conclude that the NT–AuNPs have been modified with the inorganic–biorganic composite. An interesting point is to recover the modified nanoparticles in solution by changing the applied potential. For this purpose desorption of the melanin–iron modified NT–AuNPs from the HOPG substrate was induced by performing voltammetric cycles between −0.2 V and −1.6 V in 0.1 M NaOH aqueous solution.[2] Then, this electrolyte was kept in contact with hexane for 24 h in order to extract the modified AuNP. Afterwards, a clean HOPG sample was immersed in the hexane solution for 24 h, then carefully rinsed, dried under nitrogen, and finally analyzed by XPS (Figures 1 a–d, red dots). We can observe the same Au, S, Fe and N signals again, and also a small amount of oxidized S species (binding energies > 165 eV) produced during the prolonged exposure of

Figure 2. "Voltammogram" (or cyclic voltammogram) (first scan) recorded at $v = 0.025$ Vs^{-1} in 0.1 M NaOH for melanin–iron deposited on NT–AuNP.
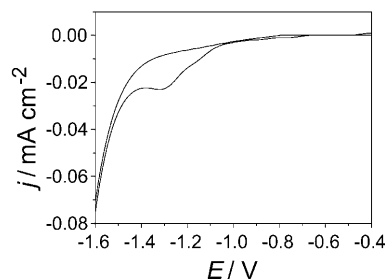

Figure 3. Current density–potential profile corresponding to NT reductive desorption from the NT–AuNPs supported on HOPG. Electrolyte: 0.1 M NaOH. Scan rate: 0.05 Vs^{-1}.

the sample to oxygen and light.[15] It should be recalled that no traces of Fe and N are observed when performing the same procedure with a clean HOPG in contact with AuNP-free melanin solutions. These results demonstrate that the melanin–iron modified NT–AuNPs are delivered to the NaOH solution by a simple change in the applied potential and they are then recovered in hexane solution by extraction.

TEM is an appropriate technique to independently determine the Au core size because the biopolymer cannot be observed due to the low contrast of the organic matter to the electron beam. Note that the iron content in the melanin polymer is also too small to be detected in the HRTEM images.

TEM images and their size distribution plots from original NT–AuNPs, NT–AuNPs adsorbed and delivered from HOPG surface, and modified and delivered melanin iron NT–AuNPs are shown in Figures 4 a,b,c respectively. Results show that the
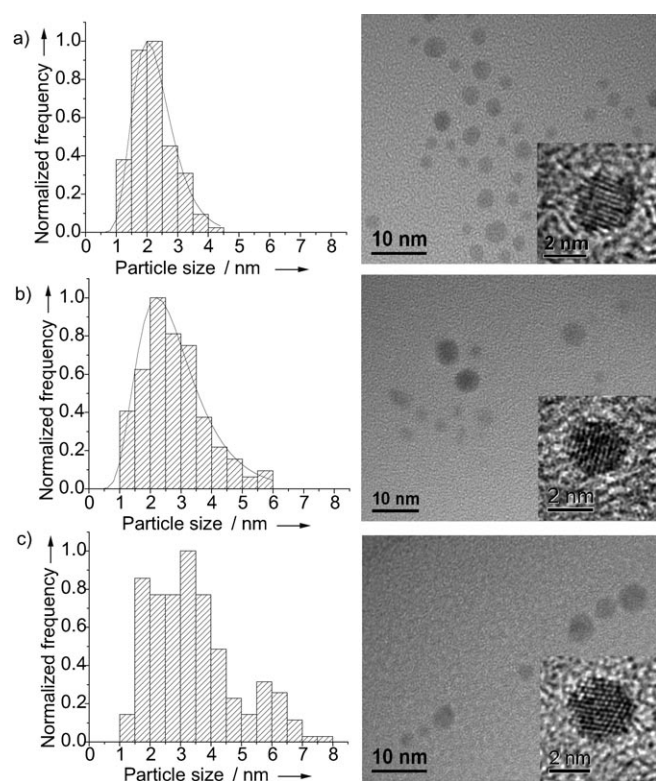

Figure 4. TEM image and size distribution functions of a) the original NT–AuNPs, b) NT–AuNPs after adsorption and release from the HOPG surface, and c) melanin–iron covered NT–AuNPs after release from the HOPG surface. Solid lines in (a) and (b) are the fitting of experimental data using a Log-normal distribution. Insets in the TEM images show detail of the Au atomic arrangement.

size distribution function of the melanin–iron modified NT–AuNPs becomes broader, although the average size remains constant (≈ 3 nm) and very close to the original and adsorbed–desorbed NT–AuNPs. This means the modification procedure doesn't significantly alter the AuNP identity.

On the other hand, it is well-known that SAXS can be used to estimate the shape and average size of metallic nanoparti-

cles, proteins[16] and melanins,[17] being sensitive in our case to both the Au core and its capping biopolymer. Therefore, we have made SAXS measurements on the AuNPs before and after being modified with melanin–iron to determine the effect of the melanin deposit on the NT–AuNPs. Figure 5 shows the
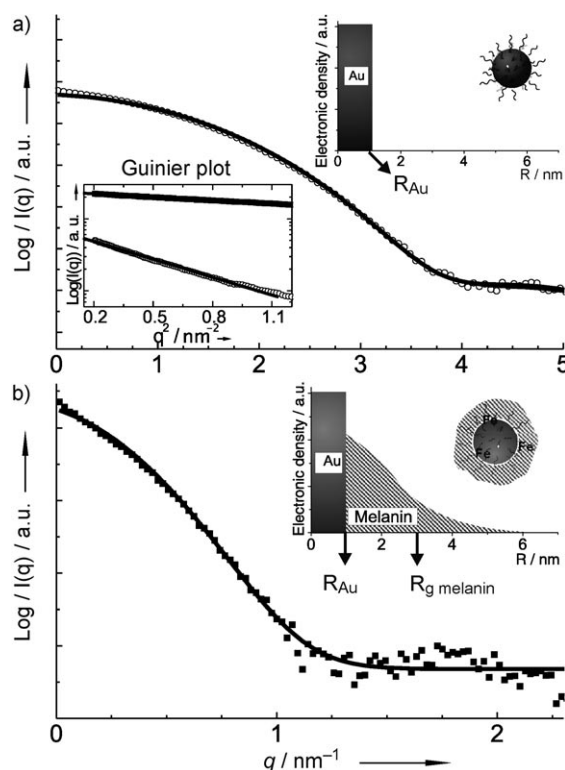

Figure 5. SAXS curves obtained for a) NT-AuNP and b) melanin–iron NT–AuNP. Inset: Guinier plot for both samples and the linear fit. Right upper corner: scheme of the electronic density profile used for fitting procedure.

dispersion curves as a function of the modulus of the scattering vector q obtained for the NT–AuNPs (a) and melanin–iron NT-AuNPs (b) in hexane solution. The change in the shape of both curves provides clear evidence that the NP modify their size after being modified with melanin.

In order to make an estimation of the change in the NP size we have made a Guinier plot,[18] shown in the inset in Figure 5. The change in the slope α_G in the Guinier plot indicates an increase in NP average gyration radius in melanin-modified particles. The radius of gyration $R_g = \sqrt{3\alpha_G}$ of an homogeneous and spherical object is related to its geometrical radii R by $R_g = \sqrt{3/5}R$ (see Supporting Information). To better understand these changes we have fitted the dispersion curves from NT–AuNP assuming that the nanoparticles had a spherical shape and a Log normal size distribution, as suggested by TEM data in Figure 4. This procedure gives a size distribution consistent with that obtained by TEM (see Figure 5 a and the Supporting Information). In the case of the melanin iron NT–AuNPs we propose a two-density model, one constant profile attributed to the AuNP core and a Gaussian electronic density that is attributed to the melanin polymer shell (see Figure 5 b). The obtained gyration radius of the Gaussian electronic density

shell is $R_{gMelanin} = 2.89(7)$ nm ($R = 3.75$ nm) and an average Au core gyration radius of $R_{gAu} = 1.03(1)$ nm ($R = 1.33$ nm) which is consistent with the size of the Au core from TEM data.

Finally, we discuss the possible mechanism related to the functionalization and delivery of the AuNP. In a previous paper[2] we showed that the thiol-capped AuNP are adsorbed on HOPG forming islands two or three layers in height. These islands are stabilized by hydrophobic forces, chain-substrate, and chain–chain interactions (between AuNP layers). During polarization at −1.0 V disorder and a small desorption of thiols take place, favoring the incorporation of small melanin oligomers and iron at these sites. As melanin is a conducting polymer the NT–AuNP could be practically covered by melanin–iron. However, it is reasonable to assume that the NT-capped AuNPs interface in contact with the HOPG is not covered by the melanin-iron layer. We speculate that the thiol reductive desorption starts at the first adsorbed AuNP layer decreasing dramatically the chain–chain interactions and interdigitation that connect the outer iron-modified AuNPs layers to the first AuNP layer, that is, promoting the delivery of a significant amount of nanoparticles.

Conclusions

We have developed a new strategy that allows the capture, chemical modification, and delivery of small metallic nanoparticles to a preset environment. The methodology consists of i) adsorption of thiol capped-AuNPs on carbon substrates, ii) electrochemical modification iii) delivery of the modified nanoparticles by applying a small potential signal. Melanin–iron modified NT–AuNPs have been used as a model system to explore this simple strategy.

Experimental Section

Gold nanoparticles (AuNPs) 2.7 nm in diameter capped with nonanethiol (NT) were prepared by the Brust method.[19] The thiol-capped AuNPs were immobilized by immersing HOPG substrates (24 h) in AuNP solutions in hexane (2 mg mL^{-1}), as previously described[2].

Melanin films were electrochemically self-assembled as described in ref. [8]. The AuNPs supported on the HOPG surface were immersed in a synthetic eumelanin-containing aqueous 0.1 M NaOH solution (0.3 g L^{-1}, melanin from Sigma, M8631). This solution also contains ~0.3 ppm of Fe, as determined by atomic absorption spectroscopy. The AuNPs–HOPG substrate was polarized at −1.0 V in a conventional three-electrode electrochemical cell containing the melanin solution for 2 h in order to coat the AuNP with a melanin-iron cap. In all electrochemical measurements a Ag/AgCl sat'd KCl electrode and a platinum foil were used as reference and counter electrode, respectively.[2] The reductive electrodesorption of NT and the cyclic voltammetry of the melanin-iron AuNPs on HOPG deposit were performed in a deareated 0.1NaOH M solution.

The samples were characterized by X-ray photoelectron spectroscopy (XPS) using a Mg K source (XR50, Specs GmbH) and a hemispherical electron energy analyzer (PHOIBOS 100, Specs GmbH). The sizes of the AuNPs dispersed in hexane before and after modification were determined by high resolution transmission electron microscopy (HRTEM) using a Philips CM200 equipment, and small angle X-ray scattering (SAXS) at the SAXS-2 beamline of the Laboratorio Nacional de Luz Sincrotron (LNLS, Campinas, Brazil) using the Guinier approximation.[18] The scattering intensity curves were measured using CCD as function of the modulus of the scattering vector [$q = 4\pi/\lambda$ sin ($\epsilon/2$)], ϵ being the scattering angle and $\lambda = 0.17556$ nm the wavelength of the X-ray beam. Parasitic scattering from collimation slits was subtracted from the measured signal. The results were analyzed by using the Guinier equation.[18]

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