Journal of Colloid and Interface Science 482 (2016) 105–111

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

Gold nanoparticles-decorated silver-bipyridine nanobelts for the construction of mediatorless hydrogen peroxide biosensor

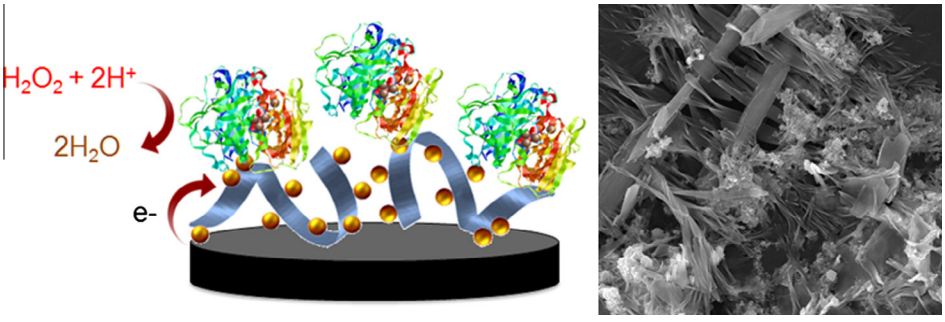
Abderrahmane Boujakhrout [a], Paula Díez [a,*], Alfredo Sánchez [a], Paloma Martínez-Ruíz [b], José M. Pingarrón [a], Reynaldo Villalonga [a,*]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain
[b] Department of Organic Chemistry I, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain

HIGHLIGHTS

- Novel gold nanoparticles decorated silver:bipyridine nanobelts are reported.
- Decorated nanobelts showed high electrocatalytic activity toward H_2O_2.
- A sensitive nanostructured enzyme biosensors for H_2O_2 is reported.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 March 2016
Revised 27 July 2016
Accepted 28 July 2016
Available online 29 July 2016

Keywords:
Biosensor
Gold nanoparticles
Peroxidase
Nanobelts
Hydrogen peroxide

ABSTRACT

Au nanoparticles modified with 4-mercaptopyridine and 6-mercapto-1-hexanol were used as coordination agents to prepare a novel hybrid nanomaterial with Ag:4,4′-bipyridine nanobelts. This nanohybrid was employed to modify glassy carbon electrodes and to construct a horseradish peroxidase-based mediatorless amperometric biosensor for H_2O_2. The electrode, poised at −100 mV, exhibited a rapid response within 4 s and a linear calibration range from 90 pM to 6.5 nM H_2O_2. The biosensor showed a high sensitivity of 283 A/M cm^2 and a very low detection limit of 45 pM at a signal-to-noise ratio of 3. The enzyme biosensor showed high stability when stored at 4 °C under dry conditions, retaining over 96% and 78% of its initial activity after 15 and 30 days of storage at 4 °C, respectively.

1. Introduction

Electrochemical enzyme biosensors are widely employed as highly selective analytical tools for biomedical, food quality assurance, clinical diagnosis and environmental monitoring [1,2]. In general, most of these biosensors devices need the use of electrochemical mediators to act as artificial electron transferring agents between the enzyme redox centre and the electrode surface. These mediators allow reduction of the overpotential and, accordingly, minimization of potential interferences during measurement [3,4]. However, the use of electrochemical mediators implies somewhat complex electrode architectures, or even worse, the addition of these mediators to the measuring solution. Therefore, the design of novel nanostructured 3D transducer elements with improved electron transfer and electrocatalytic properties is a key factor to assemble simple, reliable and cost-effective third-generation enzyme biosensors.

* Corresponding authors.
E-mail addresses: paula.diez@ucm.es (P. Díez), rvillalonga@quim.ucm.es (R. Villalonga).

http://dx.doi.org/10.1016/j.jcis.2016.07.074
0021-9797/

Gold nanoparticles (AuNP) have been extensively employed in the preparation of electrochemical enzyme biosensors due to their unique characteristics such as high electroconductivity, surface energy and surface-to-volume ratio, electrocatalytic properties, ability to decrease proteins-metal particles distance, and the possibility to act as electroconductive wires between the electrode surface and the enzyme active site allowing direct electron transfer through a tunnelling mechanism [5]. In addition, tailor-made combination of AuNP with other micro- and nanosized materials has provided a large variety of advanced hybrid nanomaterials and nanocomposites with new and synergic properties for biosensing purposes [6–8].

AuNP have been directly employed as transducer elements in enzyme biosensor by modification of the electrode surface via covalent attachment, electrodeposition, electrostatic adsorption, electropolymerization or supramolecular association [9–12]. On the other hand, a great variety of functional biosensor interfaces has been designed by combining gold nanoparticles with carbon nanotubes [6,13], graphene derivatives [14,15], electroconductive polymers [7], dendrimers [16], metal and metal oxide nanostructures [12,17,18], polysaccharides [19], etc.

In this work we describe the synthesis of original bi-functionalized AuNP capped with 4-mercaptopyridine and 6-mercapto-1-hexanol residues, and its further use to prepare novel AuNP-decorated coordination polymer nanobelts by reaction with 4,4′-bipyridine and Ag^+ ions. This nanohybrid was successfully employed as wiring material for horseradish peroxidase (HRP, EC 1.11.1.7) to construct a highly sensitive mediatorless enzyme biosensor for hydrogen peroxide. The rational of this research is supported by the well-known electrocatalytic properties of AuNP [20,21] and silver:bipyridine coordination polymers-based nanostructures [22], allowing us to envision a synergic electrocatalytic capacity for the resulting nanohybrid.

On the other hand, hydrogen peroxide was selected as target analyte due to its function as essential mediator in biology, medicine and chemistry, as well as its environmental impact as industrial waste. In addition, H_2O_2 is a by-product of highly selective oxidases commonly employed in enzyme biosensor design [23].

2. Materials and methods

2.1. Reagents and apparatus

Horseradish peroxidase (HRP, Type II, 10^5 U/mg), $HAuCl_4$, $NaBH_4$, $AgNO_3$, 4-mercaptopyridine, 6-mercapto-1-hexanol and 4,4′-bipyridine were purchased from Sigma (USA). All other chemicals were of analytical grade.

Transmission electron microscopy (TEM) and high resolution field emission scanning electron microscopy (FE-SEM) were performed with JEOL JEM 2100 and JEOL JSM-6335F microscopes, respectively (JEOL Ltd., Japan). X-ray photoelectron spectroscopy (XPS) analysis was performed with a SPECS GmbH electron spectroscopy system provided with a PHOIBOS 150 9MCD analyser. Amperometric measurements were performed with an Inbea potentiostat (Inbea Biosensores S.L., Spain). Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were performed using a FRA2 μAutolab Type III potentiostat/galvanostat (Metrohm Autolab B.V., The Netherlands). A conventional three-electrode system was employed in the electrochemical studies, where the working electrode was a glassy carbon electrode (GCE, 3.0 mm diameter) modified with the nanobelts and the immobilized enzyme. An Ag/AgCl/KCl (3 M) and a Pt wire were used as reference and counter electrodes, respectively. The measurements with the biosensor were carried out at 25 °C in 0.1 M sodium phosphate buffer, pH 6.0 (working volume 10 mL). The solution was exhaustively de-aerated before each electrochemical experiment. The solutions were stirred at 300 rpm with a magnetic bar during amperometric measurements. For analytical purposes, 1 μM H_2O_2 solutions in 50 mM sodium phosphate buffer, pH 6.0 were freshly prepared.

2.2. Synthesis of the functionalized AuNP

Bi-functionalized AuNP were prepared through a modification of our previously reported protocol [6,10]. Briefly, 50 mg of $HAuCl_4$ were dissolved in 12.7 mL of de-aerated DMSO. This solution was added dropwise to other 12.7 mL of de-aerated DMSO containing 60 mg sodium borohydride, 10 mg 4-mercaptopyridine and 36.2 mg 6-mercapto-1-hexanol under vigorous stirring. The reaction mixture turned deep brown immediately, but the reaction was continued for 24 h. The functionalized AuNPs were then precipitated by adding 25 mL CH_3CN, collected by centrifugation, and washed with 50 mL CH_3CN:DMSO (1:1 v/v), 50 mL ethanol and 10 mL diethyl ether. The nanoparticles were finally isolated by centrifugation and dried under N_2.

2.3. Preparation of the AuNP-NB hybrid nanomaterial

This hybrid nanomaterial was prepared by modification of the protocol reported for silver:bipyridine nanobelts [22]. Stock solutions of 10 mg/mL AuNP and 34 mg/mL $AgNO_3$ in double distilled water, and 13 mg/mL 4,4′-bipyridine in ethanol were freshly prepared. To prepare the AuNP-NB nanohybrid, 200 μL of AuNP solution were mixed with 1 mL $AgNO_3$ and 2 mL 4,4′-bipyridine stock solution under continuous stirring at room temperature. After 10 min, the resulting purple precipitate was centrifuged, two-times washed with ethanol and finally dispersed in ethanol up to 14 mg/mL final concentration.

2.4. Preparation of the nanostructured enzyme electrode

A polished GCE was first coated with the nanohybrid material by depositing 20 μL of the AuNP-NB ethanolic dispersion on the electrode surface and allowing drying. HRP was further immobilized on the nanostructured electrode by dropping 12 μL of a 10 mg/mL enzyme solution in 100 mM sodium phosphate buffer, pH 6.0, and mixed with 2 μL of 25% (v/v) glutaraldehyde. The electrode was kept at 4 °C for 1 h, then washed several times with cold 50 mM sodium phosphate buffer, pH 6.0, dried and finally stored in refrigerator until use (HRP/AuNP-NB/GCE).

3. Results and discussion

The steps involved in the preparation of the hybrid nanomaterial and the HRP enzyme electrode is illustrated in Scheme 1. Gold colloids were first grown in the presence of two different thiol ligands, 4-mercaptopyridine and 6-mercapto-1-hexanol, yielding dark red[1] and water soluble nanoparticles. The bi-functionalized AuNP particles showed spherical geometry with an average diameter of 2.7 ± 0.9 nm, as determined by TEM (Fig. 1A). The radial geometry and small size exhibited by the nanoparticles resulted from the synthetic approach used, in which both metal reduction and attachment of thiolated ligands to the surface of the developing Au particles take place in the same step [10,24]. The size histogram for the AuNPs is reported in Fig. 1S (Supporting Information).

The selection of the two different thiol-derivatives used as capping ligands for the AuNPs was rational made according to the

[1] For interpretation of color in Scheme 1, the reader is referred to the web version of this article.