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Direct electrochemistry of hemoglobin and biosensing for hydrogen peroxide using a film containing silver nanoparticles and poly(amidoamine) dendrimer

Marina Baccarin [a,b], Bruno C. Janegitz [b,c,*], Rodrigo Berté [a], Fernando Campanhã Vicentini [b], Craig E. Banks [d], Orlando Fatibello-Filho [b], Valtencir Zucolotto [a]

[a] Nanomedicine and Nanotoxicology Group, Instituto de Física de São Carlos, Universidade de São Paulo, 13566-390 São Carlos, SP, Brazil
[b] Departamento de Química, Universidade Federal de São Carlos, 13565-970 São Carlos, SP, Brazil
[c] Departamento de Ciências da Natureza, Matemática e Educação, Universidade Federal de São Carlos, 13600-970 Araras, SP, Brazil
[d] Faculty of Science and Engineering, School of Chemistry and the Environment, Division of Chemistry and Environmental Science, Manchester Metropolitan University, Chester Street, Manchester M15GD, UK

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ABSTRACT

A new architecture for a biosensor is proposed using a glassy carbon electrode (GCE) modified with hemoglobin (Hb) and silver nanoparticles (AgNPs) encapsulated in poly(amidoamine) dendrimer (PAMAM). The biosensors were characterized using ultraviolet–visible spectroscopy, ζ-potential and cyclic voltammetry to investigate the interactions between Hb, AgNPs and the PAMAM film. The biosensor exhibited a well-defined cathodic peak attributed to reduction of the Fe^{3+} present in the heme group in Hb, as revealed by cyclic voltammetry in the presence of O_2. An apparent heterogeneous electron transfer rate of 4.1 s^{-1} was obtained. The Hb–AgNPs-PAMAM/GCE third generation biosensor was applied in the amperometric determination of hydrogen peroxide over the linear range from 6.0×10^{-6} to 9.1×10^{-5} mol L^{-1} with a detection limit of 4.9×10^{-6} mol L^{-1}. The proposed method can be extended to immobilize and evaluate the direct electron transfer of other redox enzymes.

1. Introduction

Hemoglobin (Hb) is a metalloprotein with a quaternary structure that contains four polypeptide chains (globin chains) and one heme group bound to each of the globin chains. It is present in red blood cells and has utmost importance to humans, since it carries oxygen throughout the body via the circulatory system [1]. Hb presents a metal cation (iron) bound to the porphyrin and due to its reported beneficial properties, efforts have been conducted to develop biosensors using Hb for the determination of hydrogen peroxide (H_2O_2) [1,2]. In this regard, third-generation biosensors based on Hb and direct electron transfer between the redox couple of the active center of the biomolecule and the electrode surface have been proposed. Such biosensors offer advantages such as high sensitivity and selectivity as they operate closer to the potential of the enzyme, and therefore reducing the signal from interfering reactions/species [3–5].

Nanomaterials present distinct characteristics, such as good mechanical strength and high electrical conductivity [6], which have attracted great interest to the scientific community [7]. Such properties combined with the potential applications of nanotechnology have made such materials very attractive for the development of electrochemical sensors [8–12] and biosensors [13–16]. Indeed, stable dispersions have been prepared with nanomaterials for electroanalysis [17,18], which provide good stability and do not interfere in the electron transfer at electrode/solution interface. As examples, we can highlight the use of metal nanoparticles (MNPs) [18–21], graphene [2,22] and/or carbon nanotubes [23,24].

Modified electrodes with MNPs such as gold, silver, palladium, copper and nickel nanoparticles present advantages over the use of macroelectrodes, including the larger contact area, increased mass transfer and electrocatalysis [25]. Silver nanoparticles (AgNPs) are used in the manufacture of consumer products, such as textiles, personal hygiene, food storage containers, appliances, paint and even dietary supplements due to their antimicrobial effects [26]. Moreover, AgNPs have been used in the development of new electrochemical sensors and biosensors [27–29].

Dendrimers are macromolecules with high molecular weight synthesized by sequentially radial growth from a polyfunctional core. The number of monomer units incorporated into each layer is, successively, doubled or tripled compared to the previous layer. As a result, a highly branched structure is created with a large number of functional groups on its surface, e.g. poly(propyleneimine) and poly(amidoamine)

* Corresponding author at: Departamento de Ciências da Natureza, Matemática e Educação, Universidade Federal de São Carlos, 13600-970 Araras, SP, Brazil.
E-mail address: brunocj@ymail.com (B.C. Janegitz).

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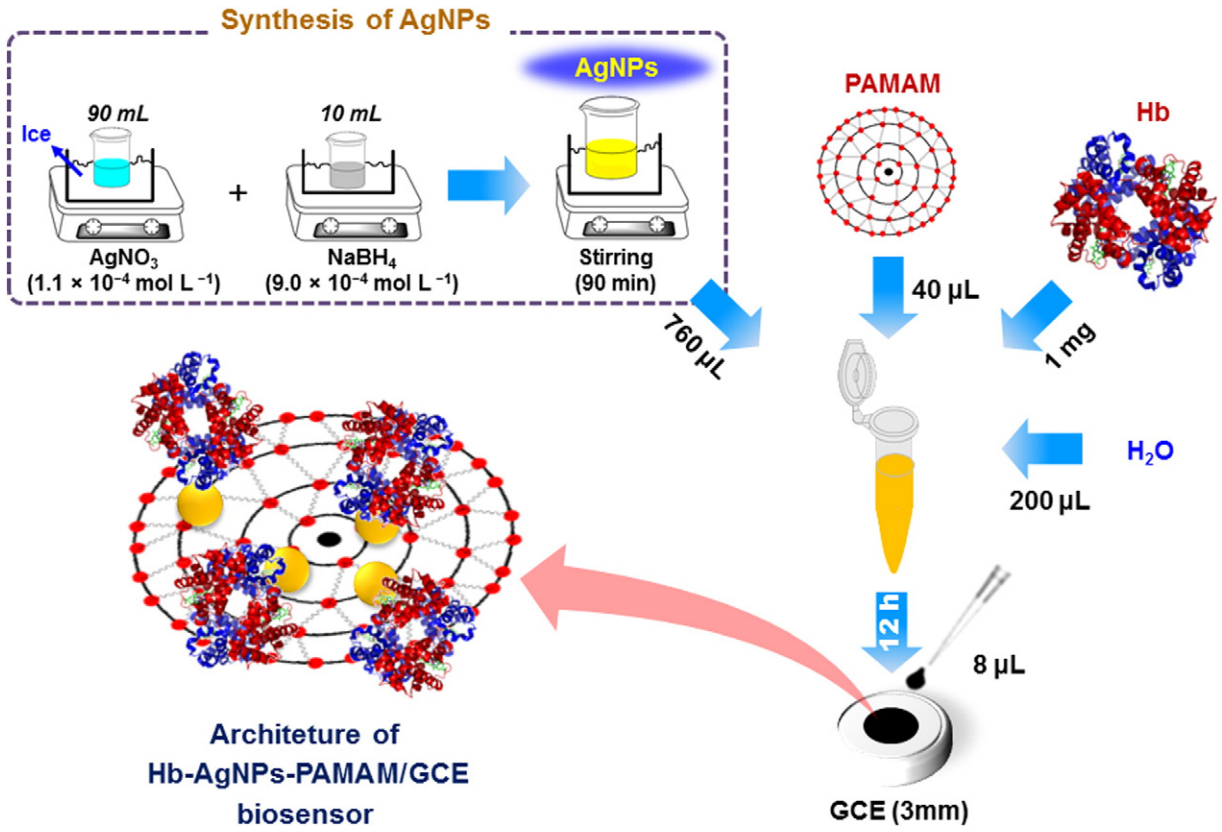

Fig. 1. Schematic representation of Hb–AgNPs-PAMAM/GCE biosensor fabrication process.

(PAMAM). PAMAM is a dendrimer consisting of alkyl diamine core and branches of tertiary amines, which are applied in different areas such as medicine, genetics, biotechnology and cosmetics [30,31]. PAMAM can be used for immobilization of biological materials through covalent and/or electrostatic bonds between their amino/hydroxyl groups and peripheral amine groups in the enzyme.

Herein, we report a new architecture for the fabrication of third-generation biosensors using Hb, AgNPs and PAMAM immobilized on the surface of glassy carbon electrode (Hb–AgNPs–PAMAM/GCE), resulting in a device easy to construct, that exhibits fast response and high sensitivity and can be applied to the determination of H_2O_2.

2. Experimental

2.1. Chemicals

Silver nitrate, sodium borohydride, PAMAM and bovine Hb were obtained from Sigma-Aldrich. All other reagents were of analytical grade and were used as received. All solutions were prepared with ultrapure water (resistivity > 18.0 MΩ cm) obtained from a Millipore Milli-Q system (Billerica, USA). A 0.1 mol L^{-1} of phosphate buffer (pH 7.0) was used as the electrolyte solution, which was prepared with Na_2HPO_4 and NaH_2PO_4 salts from Sigma-Aldrich.

2.2. Instruments

Scanning electron microscopy (SEM) images were obtained with a Supra 35-VP microscope (Carl Zeiss, Germany), with an electron beam energy of 25 keV. Histogram was constructed using the public-domain Image J image-processing software. A Hitachi U2001 spectrometer was used for measurements of UV–vis spectroscopy and a Zetasizer Nano ZS (Malvern) was employed to investigate the interactions between Hb, the AgNPs and PAMAM. Electrochemical measurements were performed using an Autolab PGSTAT12 (Eco Chemie) potentiostat/galvanostat coupled to a microcomputer managed by 4.9 GPES software. A conventional three-electrode system, using the biosensor Hb–AgNPs–PAMAM/GCE as a working electrode, a platinum plate as a counter electrode, and a Ag/AgCl (3.0 mol L^{-1} KCl) as a reference electrode completing the circuit was employed for electrochemical studies.

2.3. Synthesis of silver nanoparticles

An aliquot of 90 mL of 1.1×10^{-4} mol L^{-1} $AgNO_3$ stock solution and 10 mL of 9.0×10^{-4} mol L^{-1} $NaBH_4$ solutions was respectively transferred in two flasks. Each solution was stirred individually for 20 min in an ice bath. The solutions were mixed to obtain a final concentration of 9.9×10^{-5} mol L^{-1} $AgNO_3$ and 9.0×10^{-5} mol L^{-1} of $NaBH_4$. This mixture was stirred for 90 min in an ice bath. The final product (AgNPs), a yellow solution, was stored in an amber vial, protected from light, at room temperature for further use. The formation of AgNPs was confirmed by UV–vis spectrometry. For this, 100 µL of stock solution was diluted in water (1.0 mL) and the sample was analyzed.

2.4. Preparation of Hb–AgNPs-PAMAM/GCE biosensor

The GCE (Ø = 3.0 mm) was polished with alumina for 5 min to obtain a mirror-like surface on the electrode and then washed with ultrapure water. A mass of 1.0 mg of Hb was dispersed in 760 µL AgNP solution. Following, 40 µL of a 6.6×10^{-2} mol L^{-1} PAMAM and 200 µL of ultrapure water were added to this suspension. The resultant mixture was stirred for 30 min for homogenization. Next, 8 µL of the Hb–AgNPs-PAMAM dispersion was dropped onto the GCE surface and the solvent was evaporated at 4 °C in a desiccator for 12 h. For comparison,