Sensors and Actuators B 181 (2013) 136–143

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

In situ electrodeposition of biocomposite materials by sinusoidal voltages on microelectrodes array for tyrosinase based amperometric biosensor development

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ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 18 January 2013
Accepted 27 January 2013
Available online 4 February 2013

Keywords:
Amperometric biosensor
Sinusoidal voltages
Electrochemical impedance spectroscopy
Microelectrode arrays
Dopamine
Catechol
Tyrosinase

ABSTRACT

The amperometric detection of dopamine (DA) and catechol (CT) was achieved at tyrosinase (Ty)-poly(3,4-ethylenedioxythiophene) (PEDOT) bio-composite film-modified gold (Au) disc microelectrode arrays. The PEDOT-Ty bio-composite coatings were prepared by electrochemical polymerization of the corresponding monomer 3,4-ethylenedioxythiophene (EDOT) in the presence of various amounts of Ty on Au microelectrode arrays using sinusoidal voltages. The use of sinusoidal voltages in preparing bio-composite coatings is reported here for the first time. The bio-composite coatings deposited on different arrays by sinusoidal voltages and potentiostatic methods were compared. The bio-composite coatings were characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), infrared reflection absorption spectroscopy (IRRAS), and atomic force microscopy (AFM). This new preparation procedure provided a simple and reliable enzyme immobilization preserving the enzymatic activity. The amperometric detection of DA and CT was achieved at a working potential of -0.2 V in physiological buffered solutions. Multianalyte detection was achieved connecting different microelectrode arrays to a bipotentiostat. The analytical performance of the biosensor in terms of limit of detection, linear response range, sensitivity, and working stability, were investigated. The microelectrodes array based biosensor obtained by using sinusoidal voltages displayed a linear response for dopamine concentrations ranging from 10 to 150 μM, with a limit of detection (3 Sb/m, where Sb is the standard deviation of the blank and m is the slope of the calibration plot) of 0.99 μM. A linear response for catechol concentrations ranging from 10 to 60 μM and a detection limit of 5.58 μM were also obtained for this micro-biosensor.

1. Introduction

There is currently a growing demand for continuous, fast, selective and sensitive monitoring of compounds of biological and environmental significance. Multianalyte sensors based on electrochemical transducers are very promising tools in this context. Multianalyte detection represents an emerging trend in the field of chemical sensors. The integration of technological developments, and in particular of a new generation of smart and composite materials interacting with their surrounding, is bringing huge potential for the development of new sensors. The area of electrochemical sensors has greatly benefited from the development of micro and nano-technologies. Microelectrodes (MEs) have critical dimensions less than the scale of a diffusion layer, and they have enabled the measurements of fast electron transfer kinetics into previously inaccessible domains of time, space and media [1,2]. MEs can also be wired in parallel with each microelectrode acting diffusionally independently, resulting in a signal that is typically orders magnitude larger compared to a single microelectrode. This device, called array, can contain many MEs in both regular and random distributions [2–8]. On the other hand, conducting polymers (CPs) have drawn considerable interest in recent years because of their potential applications in different fields such as sensors [9,10], electrochemical displays [11], and catalysis [12,13]. Among the organic conducting polymers, poly(thiophene) and its derivatives, such as poly(3,4-ethylenedioxythophene), (PEDOT), have attracted particular interest due to their electrochemical properties and ability to incorporate inorganic and bio/organic components [14–17] but it can be concerns metallic particles [18] as well as organic molecules [19–21]. CPs can be deposited as dense modifying

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http://dx.doi.org/10.1016/j.snb.2013.01.060

layers on common electrode substrates, which is of importance to the development of many technologies, including electrochemical sensor technology. Recently, it has been demonstrated that CPs can provide a suitable microenvironment for enzymes immobilization, retaining the enzymatic activity [22,23]. Tyrosinase (Ty, polyphenol oxidase, E.C. 1.14.18.1) is an enzyme that, in the presence of oxygen, catalyses the hydroxylation and oxidation of monophenols to o-quinones (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) [24]. The o-quinone can be then reduced at the electrode surface at low potentials giving o-diphenol, this being the functioning principle of several electrochemical biosensors [25–29]. A major problem of electrochemical sensors employing Ty is the difficulty in achieving long-term stability because of the loss of enzymatic activity following enzyme immobilization. Ty immobilization during the electrochemical polymerization of pyrrole [30,31], aniline [32], thiophene [33] and their copolymers [34], was proved to lead to more stable biosensors. However, only a limited work on the direct entrapment of Ty during the electrochemical polymerization of 3,4-ethylenedioxythophene (EDOT) via potentiostatic methods has been reported [35–37]. Therefore, our interest focused on the use of a commercially available monomer, EDOT, for the design of new electrochemical multianalyte biosensors. Furthermore, the electrochemical polymerization of EDOT monomer in aqueous solutions provides a feasible route for Ty immobilization retaining its enzymatic activity for long-term stability.

The aim of this work is the preparation of new electrochemical micro-biosensors based on composite materials containing CPs-Ty. The originality of this work lies in the in situ electrochemical deposition of both PEDOT component and Ty biological element by using sinusoidal voltages of various frequencies and amplitudes, on one hand, and in the analytical application of these new sensors in multianalyte detection, on the other. The use of sinusoidal signals for in situ immobilization of Ty into PEDOT films is not reported in the literature and it is in fact an innovative aspect in the field of electrochemical biosensors. It is worth noting that the proposed approach is related to electrochemical impedance spectroscopy (EIS). EDOT monomers were polymerized at a fixed potential in the presence of Ty. The superimposition of a sinusoidal voltage on the dc fixed potential provided a finer control of the enzyme immobilization and the porosity of the biocomposite coatings. The proper choice of the frequencies and amplitudes of the sinusoidal voltages brought an improved enzymatic activity of the immobilized Ty. The new amperometric biosensors were applied to dopamine (DA) and catechol (CT) detection in buffered aqueous solutions. The analytical performance of these biosensors was also investigated.

2. Materials and equipment

2.1. Chemicals

All chemicals: Tyrosinase (E.C. 1.14.18.1, from mushroom, 3610 units/mg solid, Sigma), dopamine hydrochloride (Fluka), catechol (Fluka), $Na_4[Fe(CN)_6] \times 10H_2O$ (Riedel-de-Haën), $K_3[Fe(CN)_6]$ (Aldrich), $LiClO_4$ (Merck), KCl (Riedel-de-Haën), KH_2PO_4 (Riedel-de-Haën), $K_2HPO_4 \times 3H_2O$ (Merck), and 3,4-ethylenedioxythiophene (EDOT, Aldrich) were used without any further purification. EDOT was used for the electrochemical preparation of the corresponding polymer. Distilled water was always used to prepare aqueous solutions.

2.2. Electrochemical measurements

The electrochemical experiments were carried out at room temperature with an Autolab potentiostat/galvanostat 302N (Ecochemie, The Netherlands) coupled to a PC running the GPES software, using a single-compartment cell with three electrodes. The electrodes used were: a gold disc microelectrode array (with microelectrodes having diameter of 20 μm and centre-to-centre separation of 50 μm) as working electrode, an Ag/AgCl/KCl (3 M) electrode as reference electrode (Metrohm), and a platinum wire (Metrohm) as auxiliary electrode. The electrochemical measurements were performed inside a Faraday cage. Before each electrochemical measurement, the surface of the working microelectrode array was electrochemically activated in 0.1 M KCl aqueous solution by holding the electrode potential at 0.0 V for 10 seconds followed by a second potential step at −2.0 V for 10 s. These potential steps were applied four times to activate the microelectrode arrays. The cyclic voltammograms were recorded simultaneously at both microelectrode arrays, using the *bipot* module of the potentiostat, in the array mode. The impedance spectra were recorded with the FRA2 module in the frequency range 100 kHz to 0.1 Hz using a sinusoidal excitation signal (single sine) with amplitude (ΔE_{ac}) of 5 mV. The electrochemical properties of the bio-composite coatings were checked by cyclic voltammetry before and after the EIS measurements. The impedance spectra were recorded at the open circuit potential (i.e. $E^{\circ'} = 0.220$ V vs. Ag/AgCl in the case of ferrocyanide–ferricyanide redox couple) in 0.1 M KCl aqueous solution containing the redox probe $Fe(CN)_6{}^{3-/4-}$, with equal concentration of the oxidized and reduced form, i.e. $C_{Ox} = C_{Red} = C = 5$ mM. The solutions for electrochemical measurements were bubbled with high purity argon, and a flow of argon was maintained over the solution during the measurements to delay re-oxygenation. The amperometric detection of DA and CT was achieved in air saturated buffered aqueous solutions at various working potentials.

2.3. Fabrication of microelectrode arrays

The microelectrode arrays used in this work comprise 816 microelectrodes of 20 μm in diameter. They are arranged in a 24 × 34 square lattice and present a centre-to-centre separation of 50 μm along the *x* and *y* axes (Fig. 1). These microelectrode arrays were fabricated on silicon chips using standard microfabrication

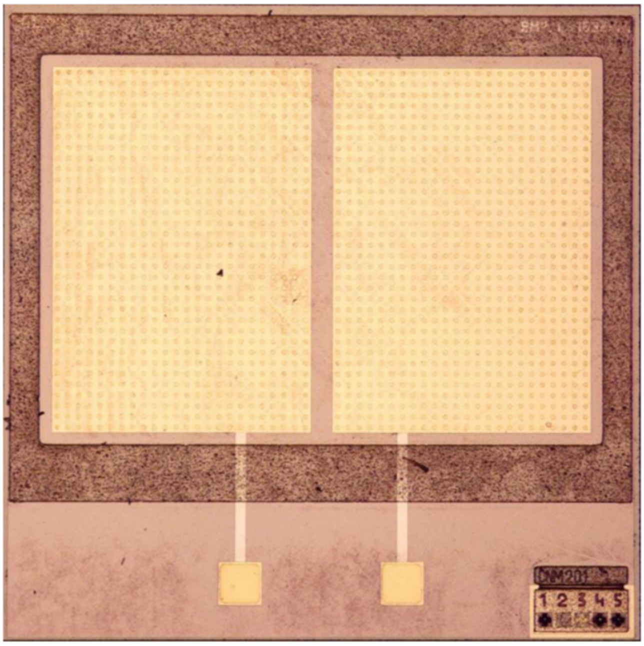

Fig. 1. Optical microscopy image of the microelectrode arrays used in this work, which comprise 816 microelectrodes of 20 μm diameter. The chip footprint is 3 mm × 3 mm.