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Monoamine oxidase B layer-by-layer film fabrication and characterization toward dopamine detection

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ABSTRACT

In this work nanostructured film composites of the monoamine oxidase B (MAO-B) enzyme, free or encapsulated in liposomes, were fabricated by the layer-by-layer (LbL) self-assembly technique, employing polyethylene imine (PEI) as polycation. Initially, the MAO-B enzyme was incorporated into liposomes in order to preserve its enzymatic structure ensuring their activity and catalytic stability. The LbL film growth was monitored by surface plasmon resonance (SPR) by gold resonance angle shift analysis after each bilayer deposition. Subsequently, the films were applied as amperometric biosensors for dopamine detection using Prussian Blue (PB) as the electron mediator. The biosensor fabricated by MAO-B incorporated into liposomes composed of DPPG:POPG in the ratio (1:4) (*w*/*w*) showed the best performance with a sensitivity of 0.86 ($\mu A\ cm^{-2}$)/($mmol\ L^{-1}$) and a detection limit of 0.33 $mmol\ L^{-1}$.

1. Introduction

Nowadays, many researches are focused on the development of new sensors with faster and sensitive diagnosis in many different areas, such as biomedical, industrial and environmental monitoring, aiming to improve the welfare and health quality. Several methods have been applied, but up to date the use of electrochemical methods with modified electrodes for sensor and biosensor applications has provided the easiness of combining fast response, easy operation, selectivity and sensitivity in low cost chemical analysis methods [1,2]. Various materials have been used in the electrode modifications toward selectivity and sensitivity enhancement, such as graphene [3,4], nanotubes [5–7], nanoparticles [8,9], conducting polymers [10,11], enzymes [12–15], etc.

Biosensors can be constructed by several methodologies, whereas the layer-by-layer (LbL) technique developed in the 90s by Decher et al. [16] has been widely used in the development of electrochemical biosensors [13,14,17] by allowing the use of the synergistic properties of different materials in a simple and versatile way, and to facilitate the control of manufacturing conditions in order to preserve the biomolecules' activity [18]. This technique allows for the immobilization of various kinds of materials such as charged biomolecules that include: DNA, enzymes, proteins and phospholipids, all using basic equipment at a relatively low cost.

Biosensors based on the immobilization of enzymes require special care to maintain their natural conformation and activity. The incorporation of liposomes is an alternative for the conservation of the biomaterial properties improving the sensitivity and the detection limit of the device [13,14,19,20].

Monoamine oxidase B (MAO-B) is a protein localized in the outer membrane of the mitochondria that catalyzes the oxidation of amines, such as dopamine and tyramine [21]. Dopamine (DA) is an important neurotransmitter of a class of molecules called catecholamine. Inadequate amounts of dopamine in our brain can lead to the development of diseases such as Parkinson's and schizophrenia [22], therefore, its quantification is extremely important for the diagnosis and monitoring of these diseases. Because of the relation of DA concentration to the occurrence of various diseases, the development of sensors for its quantification has attracted attention in recent years. Several analytical techniques have been developed for the DA determination including sophisticated methods such as mass spectrometry [23], optical spectroscopy [24–26], high performance liquid chromatography (HPLC) [27,28], and more simple and cheaper electrochemical methods [3,29–35]. Electrochemical techniques are the most exploited for DA detection, however, its quantification becomes complicated due to the presence of natural interferents such as ascorbic (AA) and uric (UA) acid, which have similar oxidation potentials to the DA [22]. The differential pulse voltammetry method applying materials capable of

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promoting the separation of the oxidation peaks of the different analytes (DA, AA and UA) has been the center of the research for DA sensors [3,32,34–36]. Here we propose an amperometric sensor using the MAO-B enzyme for specific detection of DA using PB as a mediator which allows for low potential measurements and avoids an interfering response. A range of materials has been used for the development of these devices, such as nanoparticles [33,35,37], phthalocyanines [38,39], carbon nanotubes [40,41] and graphene derivatives [3,33,36], among others.

In this study the MAO-B was immobilized in LbL films in two different ways, free or incorporated in liposomes, using polyethylene imine (PEI) as polycation. The biosensor was applied as an amperometric biosensor using Prussian Blue (PB) as an electron mediator [42]. Sensitivity, detection limit, stability and interferent tests were performed to analyze the biosensors.

2. Experimental details

2.1. Materials

Monoamine oxidase B human (MAO-B) with an isoelectric point of 5.3 and with optimal activity between pH 7.0 and 7.4, was purchased from Sigma-Aldrich. The phospholipids Dipalmitoyl phosphatidyl glycerol (DPPG) and palmitoyl phosphatidyl gliceral (POPG) were obtained from Sigma-Aldrich and Avanti Polar Lipids, respectively. The polyelectrolytes poly (ethyleneimine) (PEI), poly (vinyl sodium sulfate) (PVS) and dopamine hydrochloride (DA) were obtained from Sigma-Aldrich. The reagents were used without treatment. Solutions were prepared in a pH 7.4 0.1 mol L^{-1} potassium phosphate buffer (PBS).

2.2. MAO-B incorporation in liposomes

Liposomes were prepared as references [13,43] using a mixture of POPG and DPPG phospholipids 1:4 (*w*/*w*) (chosen after an optimization study, not shown). Briefly, phospholipids were solubilized in a chloroform:methanol mixture 8:2 (*v*/*v*) and evaporated in a rotary evaporator (IKA RV 10, HB 10, USA) at 36 °C. Then, MAO-B solution (0.2 mg mL^{-1}) was added to the lipid film and left in an ultrasound bath for 2 h for the enzyme's incorporation into the liposomes. The MAO-B solutions were analyzed by circular dichroism (CD) spectroscopy with the J-815 CD spectrometer equipment (Jasco Inc., USA).

2.3. Prussian Blue (PB) film electrodeposition onto the ITO substrates

For the electrochemical measurements ITO (tin oxide doped with indium, Delta Technologies) substrates were applied after cleaning with chloroform and isopropilic alcohol. ITO slides were modified by electrode deposition of the Prussian Blue (PB) applying a potential of +0.40 V for 100 s in a mixture of 5.0 mL of 2.0 mmol L^{-1} $K_3Fe(CN)_6$ solubilized in KCl (100 mmol L^{-1}) and 5.0 mL of 2.0 mmol L^{-1} $FeCl_3$ solubilized in HCl (10 mmol L^{-1}) [17,44]. The modified electrode was washed with ultrapure water and immersed in 100 mmol L^{-1} KCl and 10 mmol L^{-1} HCl, then an ITO/PB electrode was cycled from 0.0 to 1.0 V (vs SCE) at a scan rate of 0.05 V/s, until the voltammetric behavior stabilized.

2.4. LbL film assembly

All solutions for LbL assembly were prepared with 0.1 mol L^{-1} PBS solution pH 7.4, previously prepared from ultrapure water (18.2 MΩ cm at 25°°C). Before receiving the enzyme film, two layers of PEI/PVS were deposited to reduce the influence of surface irregularities [45]. 1.0 mg mL^{-1} PEI solution and 1.3 mg mL^{-1} PVS solution and an immersion time of 3 min for both polyelectrolytes were used. For PEI/MAO-B film assembly, we immersed the ITO/PB in a PEI solution (1 mg mL^{-1}, 3 min) and alternated by immersing in a MAO-B solution (0.2 mg mL^{-1}, either free or incorporated in liposomes, 10 min). Each deposition was followed by a washing solution (PBS) for 30 s to remove any weakly absorbed material. To analyze the LbL film growth, the SPR Navi 200 surface plasmon resonance analyzer (BioNavis, Finland) was used, with a 670 nm laser and thiol functionalized gold surface (12 h in a 150 mmol L^{-1} 11-mercaptoundecanoic acid ethanolic solution).

2.5. Electrochemical measurements

The amperometric measurements were performed with an Autolab PGSTAT 30 galvanostat/potentiostat using an electrochemical cell that consisted of a saturated calomel reference electrode (SCE), a platinum foil as an auxiliary electrode and the working electrode containing ITO/PB recovered with the free enzyme film $(PEI/(MAO\text{-}B)_7$ and liposome incorporated enzyme film $[PEI/(MAO\text{-}B/1POPG{:}4DPPG)_7$ at a potential of 0.0 V. PBS was used as support solution.

3. Results and discussions

3.1. Structural analysis of MAO-B free and incorporated in liposomes in solutions

Studies with circular dichroism (CD) give an indication of the secondary structure of proteins. Fig. 1 illustrates the CD spectra for free MAO-B and incorporated in liposomes 1POPG:4DPPG solutions in PBS. The spectrum of MAO-B incorporated in liposomes indicated two minimums of 208 nm and 223 nm and a positive band at 197 nm with a negative to positive crossover at 201 nm, characteristic of an α-helix structure [46,47]. The spectrum of the free MAO-B in the buffer showed only a minimum at 207 nm, possibly due to conformational changes in the absence of the liposome.

3.2. LbL film growth

The monitoring of the LbL film growth was performed by surface plasmon resonance (SPR). Fig. 2A shows the spectra of each deposited bilayer. The minimum angle of reflection θ due to absorption of the plasmon by the thiol-modified gold surface was 68.689°. With the addition of each bilayer, θ presented a sequential increase. Fig. 2B shows the shift in SPR angle Δθ with the deposition of the bilayer. Since the magnitude of Δθ is dependent upon the thickness and the refractive index of the deposited layers on the gold surface [48], we can believe in a linear growth behavior on the LbL films. In the presence of liposomes Δθ was higher, a fact already predicted due to the fact that liposomes are relatively large vesicles (100–1000 nm) and cause, therefore, greater changes in the surface refractive index when attached to the surface [49].

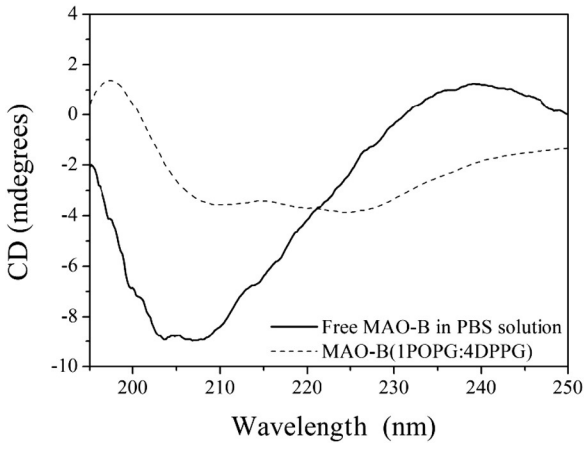

Fig. 1. CD spectra for free MAO-B and liposome incorporated MAO-B solutions in PBS.