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Glassy carbon electrode modified with horse radish peroxidase/organic nucleophilic-functionalized carbon nanotube composite for enhanced electrocatalytic oxidation and efficient voltammetric sensing of levodopa

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ABSTRACT

A novel and selective enzymatic biosensor was designed and constructed for voltammetric determination of levodopa (L-Dopa) in aqueous media (phosphate buffer solution, pH = 7). Biosensor development was on the basis of to physically immobilizing of horse radish peroxidase (HRP) as electrochemical catalyst by sol–gel on glassy carbon electrode modified with organic nucleophilic carbon nanotube composite which in this composite p-phenylenediamine (pPDA) as organic nucleophile chemically bonded with functionalized MWCNT (MWCNT-COOH). The results of this study suggest that prepared bioorganic nucleophilic carbon nanotube composite (HRP/MWCNT-pPDA) shows fast electron transfer rate for electro oxidation of L-Dopa because of its high electrochemical catalytic activity toward the oxidation of L-Dopa, more —NH_2 reactive sites and large effective surface area. Also in this work we measured L-Dopa in the presence of folic acid and uric acid as interferences. The proposed biosensor was characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), FT-IR spectroscopy and cyclic voltammetry (CV). The differential pulse voltammetry (DPV) was used for determination of L-Dopa from 0.1 μM to 1.9 μM with a low detection limit of 40 nM (for S/N = 3) and sensitivity was about 35.5 μA/μM. Also this biosensor has several advantages such as rapid response, high stability and reproducibility.

1. Introduction

Levodopa (L-3,4-dihydroxyphenylalanine, L-Dopa, LD, levodopa is abbreviated as L-Dopa in all following text) is an important neurotransmitter and a medication used to control symptoms Parkinson's disease. Parkinson's disease is associated with low levels of dopamine in the brain where dopamine is a neurotransmitter that helps control the brain's reward, learning and emotional centers. L-Dopa converted to dopamine in the body by enzymatic reaction catalyzed by dopadecarboxylase [1,2]. As can be seen, it is proved that changes in L-Dopa levels are important in effective brain process. So, accurate determination of component values of L-Dopa is important and necessary.

Various types of analytical methods have been reported for measurement of L-Dopa including capillary zone electrophoresis [3], spectrophotometry [4] and HPLC [5]. But these techniques are not sensitive and simple. Also these approaches usually are expensive, long time consuming, and low selectivity and complicate sample preparation procedures. Therefore they are not appropriate for routine analysis. Generally electrochemical detection methods have many advantages such as low cost, specific, rapid, simple operation, and high sensitivity and selectivity [6–8].

Among the electrochemical methods, applying of enzymatic biosensors is one of the simplest, most selective, accurate and sensitive methods. In addition the biosensors have some advantages such as low noise, wide detection range, fast response, high stability, and low detection limit and reproducibility; so they have been found to be of great interest [9–12]. Several biosensors for phenolic compounds or catechol amines have been reported by using purified or extracted from natural sources for example lactase [13,14], tyrosinase, laccase [15,16] and peroxidase enzyme biosensor [17,18].

However, these biosensors have some common disadvantages such as low conductivity, instability due to leaching enzyme from membrane support, low reproducibility, high response time, low stability and low sensitivity. Therefore, development of biosensor is still in need for modifiers which have high selectivity, stability, sensitivity and reproducibility for different analyte measurements in various samples. In this regard, the traditional electrodes like glassy carbon electrode (GCE), in biosensors can be modified with different compounds and materials to obtain the optimal electrochemical properties. Glassy carbon electrode is a non-graphitizing carbon with many important properties such as low density, high temperature resistance, low electrical and thermal resistance that was used as basic electrode in developing of

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many modified electrode for determination of various biological material and drugs [19,20].

Between peroxidases which were used in biosensors, horseradish peroxidase (HRP) is one of the most important enzymes that are obtained from a plant source which is commonly used in the determination of phenolic compounds because of its advantages such as low cost, easy availability and high selectivity for phenolic compounds and high purity.

There are various chemical and physical methods for immobilization of enzyme on electrode surface. One of the most common chemical immobilization methods is applying of covalent linkage using glutaraldehyde and carbodiimide [21,22]. Generally, porous networks were used for trapping of enzyme in physical immobilization methods. We used silica sol–gel as porous network for immobilization of HRP because of its advantages such as biocompatibility, large surface areas, electroinactive in aqueous medium, high permeability, high mechanical and thermal activity, nontoxicity and great ion exchange capacities.

In recent years, significant advances have occurred in all areas of nanotechnology. In other words, nanotechnology affected every aspect of human life such as drugs, power and speed of computers, energy resources and food industry [23–26]. In this regard, nanomaterials are a new class of nanoscale materials with new properties that have various applications in different fundamental fields [27–30]. Carbon nanotubes have become one of the most widely used in modification of electrodes in biosensors over the past decade because of their special mechanical, physicochemical, structural and electrical properties [31–33]. In fact, carbon nanotubes (CNTs) are tube-shaped materials that are made of carbon and have a diameter measured on a nanometer scale thus they are nanostructured. Overall, carbon nanotubes show a unique combination of high surface area, stiffness, excellent biocompatibility, strength, significant mechanical strength, fast electron transfer, tenacity and chemical stability compared to other fiber materials which usually lack one or more of these properties [34,35]. Also carbon nanotubes gained increasing applications in electrochemical modified electrodes due to their excellent electronic properties such as high sensitivity, low background current, electrocatalytic activities, reduction of overpotentials and electric conductivity [36–40].

On the other hand, carbon nanotubes have a significant role in both enzyme immobilization and promotion of electron transfer reactions of enzymes such as glucose oxidase [41], cytochrome c [42], myoglobin [43], hemoglobin [44], catalase [45] and horseradish peroxidase (HRP) [46,47].

Electrochemical oxidation of L-Dopa occurs in three steps in aqueous solutions. The mechanism of electrochemical oxidation of L-Dopa involves two electrochemical steps and one chemical step [48].

Firstly L-Dopa oxidated to o-levodopa quinine by HRP that is oxidized by H_2O_2. Generally quinones, very electrochemically reactive molecule, can be easily nucleophilically attacked. Both an electron-deficient ring and an electron donating unprotonated amine group as nucleophile in structure of o-levodopa quinine are found. Under such circumstances, intramolecular cyclization reaction of 1,4-Michael addition is done [49]. So the second step in the mechanism of electrochemical oxidation of L-Dopa is chemical step in which intramolecular cyclization via 1,4-Michael addition is performed and leads to L-Dopa quinine. In the third electrochemical oxidation step, the product of second step is easily oxidized by HRP and L-Dopa chrome is formed. However, it can be said that the presence of carboxyl group as electron density attractive group in the structure of L-Dopa and space hindrance, causes the intramolecular cyclization via 1,4-Michael addition which hardly occurred for L-Dopa. So it's better to use external nucleophile for 1,4-Michael addition reaction. In addition, the various interference species such as folic acid and uric acid do not undergo an attack by nucleophile via the 1,4-Michael addition reaction, therefore, they do not interfere the detection of L-Dopa.

Some works have been reported about the reaction between o-quinones (formed during catecholamine electrooxidation on carbon electrodes) and several nucleophiles [50–53]. Also, 1,4-Michael addition reaction between o-quinones resulting from dopamine oxidation and phenylenediamine has been reported [54].

Para-phenylenediamine (1,4-diaminobenzene), a derivative of aniline, is one of the three isomers of phenyldiamine. It is commonly used as a component in engineering of polymers and composites. It is also an ingredient in hair dyes [55,56].

For the first time, up to now, in this work we report the preparation of novel biosensor based on immobilized HRP between MWCNTs that chemically bonded to pPDA (MWCNT-pPDA) and sol–gel film on glassy carbon electrode for determination of L-Dopa. In our opinion HRP/MWCNT-pPDA composite accelerates the electron transfer rate for oxidation of L-Dopa because of its electrochemical catalytic role due to the presence of HRP as mediator and MWCNT-pPDA as nucleophilic carbon nanotube composite with high effective surface area which leads to enhancement of sensitivity and selectivity of modified biosensor. The performance of biosensor such as sensitivity, linear range of L-Dopa determined, detection limit, electrode stability and selectivity in the presence of uric and folic acid was investigated.

2. Experimental

2.1. Chemicals and reagents

L-Dopa and folic acid were purchased from Darou Pakhsh Co. (Iran). Multiwalled carbon nanotube (MWCNT) was from Neutrino (www.neunano.com, Iran). Phosphate buffer solutions (PB) (50 mM) were prepared from H_3PO_4 and its salts (NaH_2PO_4 and Na_2HPO_4) and pH values were adjusted with HCl and NaOH solutions. Tetraethyl orthosilicate (TEOS) was from Merck (Germany). Horseradish peroxidase (HRP, RZ > 320 U mg^{-1}) and paraphenylenediamine (pPDA) were purchased from Sigma Chemical Co. All the other chemicals used were of analytical grade. All the solutions were prepared with deionized water and deoxygenated by highly pure nitrogen gas through them for at least 15 min before the experiments.

2.2. Apparatus

Voltammetric measurements were performed with a computer controlled μ-Autolab modular electrochemical system (PGSTAT101, the Netherlands, www.ecochemie.nl/), driven with NOVA Software (upgrade 1.7). A conventional electrochemical cell was applied with a GC (2 mm diameter, Azar Electrode Co., Iran) as working electrode, an Ag/AgCl (saturated KCl) as reference electrode and a platinum plate as counter electrode (all from Azar Electrode Co., Iran). Scanning electron microscopy (SEM) was done by VEGA TESCAN SEM. All the electrochemical experiments were performed at room temperature in air atmosphere.

2.3. Procedures

2.3.1. Functionalization of MWCNTs

For preparation of the carboxylic group functionalized MWCNT, a process was performed as follows: MWCNT ultrasonicated in a mixture of sulfuric acid and nitric acid (3:1, v/v) for 8 h to MWCNT-COOH preparation. Then MWCNT-COOH obtained was washed with deionized water and separated by centrifugation for three times [57]. For chemically immobilization pPDA on to MWCNT-COOH, the COOH group of MWCNT-COOH was acyl-chlorinated in fact COOH was turned to COCl. So MWCNT-COOH was heated in mixture of DMF and $SOCl_2$ in 70 °C for 24 h. Finally for substitution reaction between MWCNT-COOH and pPDA, 100 mg of acyl-chlorinated MWCNT (MWCNT-COCl) was added to pPDA solution and this mixture was heated in a steam bath in 60 °C for 2 h [58]. Treated MWCNT separated from reaction solution by centrifugation and washed several times with deionized water. Obtained