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Design of a multiwalled carbon nanotube–Nafion–cysteamine modified tyrosinase biosensor and its adaptation of dopamine determination

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ABSTRACT

In this work, a multiwalled carbon nanotube (MWCNT)–Nafion–cysteamine (CA) modified tyrosinase biosensor brings a new and original perspective to biosensor technology intended for the development of dopamine determination. Dopamine measurements were done at 0.2 V with the amperometric method by the developed biosensor system. In addition, in this study dopamine determination was carried out by using the differential pulse voltammetry method between potentials of 0.4 and −0.15 V. In the optimization studies of the biosensor, some parameters such as optimal pH, optimal temperature, optimal enzyme amount, and effect of MWCNT concentration were investigated. Afterward, in the characterization studies, some parameters such as linearity and reproducibility were determined. In the reproducibility experiment, an average value of 1.026 μM, a standard deviation of ±0.03975, and a coefficient of variation of 3.8% were determined for a 1-μM dopamine concentration (n = 15). Determination of dopamine was carried out in drug samples by the developed biosensor.

Dopamine, chemically known as 3,4-dihydroxyphenyl ethylamine, is a neurotransmitter of great importance for the nervous system because it plays an important role in the communication between neurons. Changes in the dopamine level in the central nervous system have been associated with the pathogenesis of neurological syndromes such as Parkinson's disease and schizophrenia [1–3]. Thus, the quantitative determination of this neurotransmitter appears to be important for diagnosis, monitoring, and pharmacological intervention. Owing to its electrochemical activity, dopamine determination by electrochemical detection can be carried out with a fair amount of sensibility, simplicity, and high sample throughput. Nonetheless, the selectivity by using a bare electrode can be drastically decreased mainly in the presence of other biomolecules. Therefore, the reliable determination of phenolic compounds by using an electrochemical sensor is highly dependent on convenient modification [4,5]. The use of an amperometric biosensor, which combines redox enzyme reactions with electrochemical detection, has also been successfully employed to improve the sensitivity and selectivity of phenolic compound determination [6].

Tyrosinase, phenoloxidase, and catecholoxidase are members of the copper protein family. All of them have a common active site but different functions. Tyrosinases use molecular oxygen to catalyze two different enzymatic reactions: (i) the *ortho*-hydroxylation of monophenols to *o*-diphenols (monophenolase, cresolase activity) and (ii) the oxidation of *o*-diphenols to *o*-quinones (diphenolase, catecholase activity) [7,8].

Carbon nanotubes (CNTs)[1] have attracted much attention due to their high chemical stability, high surface area, unique electronic properties, and relatively high mechanical properties. As electrode materials, CNTs can be used for promoting electron transfer between the electroactive species and electrode and provide a novel method for fabricating chemical sensor or biosensor [9–12]. The ability of CNT-based electrode to provide electrocatalytic activity and to minimize surface fouling has been reported [13]. Depending on CNTs' electrochemical properties, the CNT-based sensors can be used for detecting NADH and hydrogen peroxide at a lower working potential [14,15]. Nafion, chemically known as tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid copolymer, has good electrical conductivity, good biocompatibility, excellent film forming and adhesion ability, high chemical stability, and ability to resist interferences from anions and biological macromolecules, making it a good matrix for biomolecule immobilization [16]. Recent studies have demonstrated improved electrochemical responses of ethanol and glucose determination at MWNT–Nafion nanocomposite film modified electrode [17].

In this work, we constructed a novel tyrosinase biosensor based on the cysteamine (CA)/multiwalled CNT (MWCNT)–Nafion–tyrosinase composite film as the immobilization matrix. This

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[1] *Abbreviations used:* CNT, carbon nanotube; CA, cysteamine; MWCNT, multiwalled CNT; Au, gold; CV, cyclic voltammetry; PBS, phosphate-buffered saline; DPV, differential pulse voltammetry.

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composite matrix combined the advantages of MWCNT and Nafion, which could promote the direct electron transfer of tyrosinase. The results showed that the immobilized tyrosinase almost retained its native structure and displayed high electroactivity and electrocatalytic response to dopamine. The biosensor has high stability and satisfactory reproducibility. This demonstrates that the composite matrix is suitable for the immobilization of tyrosinase and has potential application in the construction of biosensors.

Materials and methods

Apparatus

In the experiments, a PalmSens potentiostat (The Netherlands), a three-electrode system from CH Instruments (USA) that contains a CHI 101 model gold (Au) working electrode (2 mm diameter), a CHI 111 model Ag/AgCl reference electrode, and a CHI 115 model platinum wire counter electrode, Isolab P100 and P1000 automatic pipettes (Germany), a Yellow-Line magnetic stirrer (Germany), and a Nuve model thermostat (Turkey) were used.

Chemicals and reagents

Tyrosinase from mushroom (EC 1.14.18.1, 3900 U mg^{-1}), potassium ferricyanide, CA, Nafion (5% in a mixture of lower aliphatic alcohols and water), and MWCNT (>99% purity in the range of 7.0–15.0 nm diameter, 0.5–10 μM length) chemicals were purchased from Sigma Chemical (USA). All of the other chemicals were obtained from Riedel-de-Haen (USA). All solutions were prepared with twice-distilled water.

Electrode fabrication

Prior to coating, Au electrode surface was polished with alumina slurries on microfiber cloth to obtain a mirror surface. After that, it was thoroughly rinsed with double-distilled water and sonicated first in absolute ethanol and then in double-distilled water for 10 min to remove undesired adsorbed particles. At the next step, the electrode was cleaned by five successive cyclic voltammetry (CV) sweeps between −1.0 and +1.0 V in 0.1 M HNO_3.

The Au/CA modified electrode was formed by immersing the cleaned electrode into 50 mM CA aqueous solution at 37 °C temperature in darkness for 2 h [18]. After that, for removing the physically adsorbed CA, the electrode was thoroughly rinsed with water and dried with a nitrogen gas stream. Immobilization of tyrosinase and the MWCNT–Nafion composite film was carried out as follows. First, 10 μl of the 78-U/ml enzyme solution (prepared in phosphate-buffered saline [PBS], pH 7.0) was deposited on the Au/CA and let to dry. At the same time, a 0.5 wt% Nafion solution was prepared by diluting 5 wt% Nafion solution with ethanol. Then 10 mg of MWCNT was added to 1 ml of 0.5 wt% Nafion solution with the aid of ultrasonic agitation for 1 h to form a homogeneous MWCNT–Nafion solution. Au/CA/tyrosinase–MWCNT–Nafion modified electrode was formed by drop casting 6 μl of this solution onto Au/CA/tyrosinase and drying at room temperature in the air [19].

Measurements

Scheme 1 shows the measurement of principle of biosensor for the amperometric method. Dopamine measurements were done at 0.2 V with the amperometric method by the developed biosensor system. The measurements on biosensor were carried out with the determination of increasing current values directly proportional with dopamine concentration using the amperometric method. In addition, in this study dopamine determination based on reduction of product (dopamine-*o*-quinone) at the electrode surface was carried out by using the differential pulse voltammetry (DPV) method between potentials of 0.4 and −0.15 V.

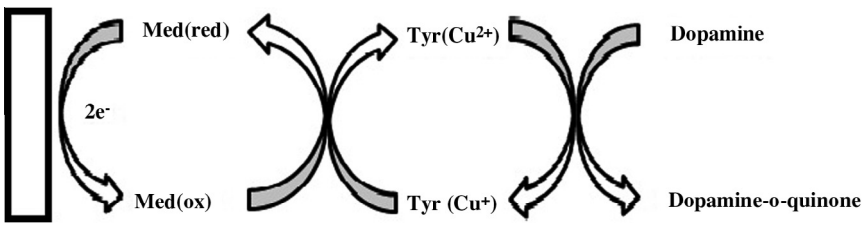
Results and discussion

Immobilization

Cyclic voltammograms were carried out at a potential range between −0.6 and 0.6 V in a phosphate buffer (50.0 mM, pH 7.0) containing 1.0 mM $Fe(CN)_6^{3-/4}$ by using bare electrode, CA-modified electrode (Au/CA), Au/CA/tyrosinase, and Au/CA/MWCNT–Nafion–tyrosinase for establishing the formation of self-assembled monolayer, immobilization of enzymes, and MWCNT–Nafion composite film. A pair of dominant redox peaks was observed on bare electrode (Fig. 1, curve a). When cysteamine monolayer formed on the electrode, a decrease was observed at cathodic (0.1 V) and anodic (0.2 V) peak currents (Fig. 1, curve b). After deposition of tyrosinase on the Au/CA, a nondistinct cathodic (0.1 V) and anodic (0.2 V) peak currents appeared (Fig. 1, curve c). Subsequently, after formation of MWCNT–Nafion, certain cathodic and anodic peak currents were observed and are attributed to the good conductive properties of the Au/CA/tyrosinase–MWCNT–Nafion modified surface (Fig. 1, curve d). The cyclic voltammograms shown in Fig. 1 were obtained with the bare electrode, Au/CA electrode, and Au/CA/tyrosinase–MWCNT–Nafion. Cyclic voltammograms showed that immobilization of MWCNT–Nafion and enzymes brought about prominent oxidation and reduction peaks that facilitated the monitoring of enzyme activity.

Response of electrocatalytic tyrosinase biosensor to dopamine

The cyclic voltammograms obtained using the Au/CA/tyrosinase–Nafion biocomposite and Au/CA/tyrosinase–MWCNT–Nafion nanobiocomposite before and after the addition of 10 μM dopamine in 50.0 mM phosphate buffer solution (pH 7.5) containing 2.50 mM $Fe(CN)_6^{3-/4}$ are shown in Fig. 2. The potential was scanned between −0.6 and 0.6 V versus Ag/AgCl at a scan rate of 50 mV s^{-1}. A pair of redox peaks was observed, with two electrodes showing the electrochemical regeneration of tyrosinase. The two electrodes show only a small background current in the absence of dopamine. On the addition of dopamine, the cyclic voltammo-



Scheme 1. Principle of the measurement by the modified tyrosinase biosensor.