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An amperometric nanobiosensor for the selective detection of K^+-induced dopamine released from living cells

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ABSTRACT

A highly sensitive amperometric sensor has been studied for selective monitoring of K^+-induced dopamine released from dopaminergic cells (PC12) which is based on an EDTA immobilized-poly(1,5-diaminonaphthalne) (poly-DAN) layer comprising graphene oxide (GO) and gold nanoparticles (GO/AuNPs). The integration of a negatively charged probe molecule on the poly-DAN/GO/AuNPs nanohybrid attained the signal enhancement to discriminate dopamine (DA) molecules from foreign species by catalytic effect and surface charge, and hydrogen bonding-based interactions with a probe molecule. The sensor performance and morphology were investigated using voltammetry, impedance spectrometry, SEM, and XPS. Experimental variables affecting the analytical performance of the sensor probe were optimized, and linear response was observed in the range of 10 nM – 1 μM with a detection limit of 5.0 nM (± 0.01) for DA. Then, the sensor was applied to monitor dopamine released from PC12 cells upon extracellular stimulation of K^+ ions. It was also confirmed that K^+-induced dopamine release was inhibited by a calcium channel inhibitor (Nifidipine). The results demonstrated that the presented biosensor could be used as an excellent tool for monitoring the effect of exogenous agents on living cells and drug efficacy tests.

1. Introduction

Label free monitoring of nerve cell excitation and release of neurochemicals in living state has fundamental significance in the better understanding of various processes implicated in neuronal functions. Among biologically interesting neurochemicals released from neuronal cells, dopamine is a vital signaling biomolecule, which plays significant role in the regulation of various physiological activities of the peripheral and central nervous systems (Hefco et al., 2003). Thus, precise monitoring of dopamine has become a critical research issue due to its low concentration in biological fluids and high clinical research significances. Previous studies have shown that an excessive stimulation of membrane bound receptors or ion channels in the dopaminergic cells trigger an abnormal neurotransmission of dopamine, which can result in the development of several psychiatric and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Schizophrenia (Montague et al., 2004; Tobler et al., 2005). It is believed that exogenous substances such as drug molecules, environmental contaminants, and toxins largely influence on the activation of dopaminergic cells as well as exocytosis of dopamine. Development of fast and label-free biosensing strategies for the observation of neuronal cell excitation and the determination of neurotransmitters dynamics using exogenous activators and inhibitors are obviously of much importance for neurobiology research.

To date, the dopamine determination from dopaminergic cell populations as well as from striatal brain slices has been carried out using conventional analytical techniques including fluorescence microscopy (Wachman et al., 2004), high-performance liquid chromatography (Cheng et al., 2000), capillary electrophoresis (Zhang et al., 2003) and spectrophotometry (Lapainis et al., 2007); however, most of these existing techniques are expensive, require sophisticated instruments to maintain and run, and the procedures for preparation of biosensors are rather time consuming. Thus, the development of rapid, facile and inexpensive analytical methods for sensitive and selective detection of trace dopamine release from living cells is very essential for routine analysis, precise clinical diagnosis of neuronal disorders and disease prevention. Thus, a PC12 cell line was used for DA release because they possess similar characteristics to that of mature sympathetic neurons (Westerink and Ewing 2008).

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Electrochemical methods have appeared to be suitable and more often employed in the clinical analysis to determine the concentration of DA owing to easy operation, cost effectiveness, and providing enough sensitivity to real time monitoring of the analytes in submicromolar concentrations (Chandra et al., 2013). Nonetheless, electrochemical observation of DA under physiological conditions is a challenging issue because its presence in the biological fluids is extremely low compared to ascorbic acid (AA) and uric acid (UA) which usually coexist with DA. Hence, the elimination of the interference caused by these species is very crucial as their oxidation potentials are almost similar, which results in overlapped voltammetric signal and the electrode often suffer from fouling effects. To overcome the influence of these factors, a variety of surface polymer modification approaches (Won et al., 2005; Abdelwahab et al., 2009; Lee et al., 2010; Chandra et al., 2013) have been exploited to enhance the sensitivity and selectivity of electrochemical techniques for dopamine detection. Despite of great performances of above mentioned techniques, improving the electrocatalytic properties of substrates for highly sensitive and target selective sensing is still considered as a challenge for bioanlytical research and medical diagnosis.

Recently, graphene oxide has attracted significant attention due to its electrical, optical, chemical and mechanical properties and many potential applications in bionanotechnology. The abundant reactive surface oxygen-containing functional groups and aromatic domains on the basal planes and edges of graphene oxide (GO) makes it to be an excellent material for biomolecular interactions (Zheng et al., 2013). In addition, Au nanoparticles (AuNPs) were often used to enhance the sensitivity of electrochemical sensors substrate (Noh, et al., 2012; Zhu et al., 2013). Thus, it is expected that the more enhanced performance can be attained when introducing of GO and AuNPs mixed composites to the conducting polymers. Since the incorporation of AuNPs and GO in the conductive polymer layer not only enhance the electronic interactions with surface polymer matrices but could also provide sufficient conductivity and large surface area, the immobilization of adequate probe molecule on the composite polymer could be readily applied for the detection of trace target species. Hence, immobilization of probe EDTA on the GO/AuNPs and polydiaminonaphthalene (polyDAN) composite was studied for the dopamine detection through the interaction between EDTA and dopamine by hydrophilic and charge interaction. To the best of our knowledge, there is no previous report on the design of GO/AuNPs/pDAN-EDTA composite film for the determination of dopamine.

In the present work, a simple, sensitive, and selective electrochemical sensor platform for the observation of dopamine released from living cells was constructed. The surface of the modified electrode was characterized by X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). The interaction between EDTA and dopamine was also simulated using energy minimized diagram. All the analytical parameters of the sensor probe were optimized and performance was evaluated for the accurate electrochemical detection of low concentration of dopamine (DA) in the presence of high concentration of interfering agents (AA and UA). Finally, the proposed biosensor was applied for the label free monitoring of K^+-induced DA exocytosis from living PC12 cells, where the influence of different concentration of extracellular K^+ ions on the membrane depolarization via the opening of voltage-dependent Ca^{2+} channels was investigated.

2. Experimental

2.1. Materials and apparatus

PC12 cells (KCLB 21721) were obtained Korean Cell Bank. Dulbecco's modified Eagle (DMEM) medium, fetal bovine serum (FBS), horse serum, trypsin EDTA, penicillin/streptomycin, 1,5-diaminonaphthalene (DAN), dopamine (DA), ascorbic acid (AA), uric acid (UA), glucose, Graphite, sulfuric acid (98%), aluminum chloride and Hydrogen peroxide (H_2O_2), Ethylenediaminetetraacetic acid (EDTA), Potassium permanganate Phosphate dibasic, sodium phosphate monobasic, Nifidipine, Sodium nitrate ($NaNO_3$), potassium chloride (KCl), $K_3[Fe(CN)_6]^{4-}$, $K_3[Fe(CN)_6]^{3-}$, $[Ru(NH_3)_6]Cl_3$, and Phosphate buffer saline solutions (PBS) were obtained from Sigma-Aldrich (USA). All aqueous solutions were prepared in ultra-pure water obtained from a Milli-Q water purification system (18 MΩ cm). All electrochemical measurements were carried out at room temperature using conventional three-electrode cell system. A modified glassy carbon with geometric area of 0.07 cm^2, Ag/AgCl (in saturated KCl), and a platinum (Pt) wire were used as the working, reference, and counter electrodes, respectively. Cyclic voltammograms (CVs) were recorded using a potentiostat/galvanostat Kosentech, model PT-1 and EG & G PAR model PAR 273A. The impedance spectra were measured with the EG&G Princeton Applied Research PARSTAT. Scanning electron microscopy (SEM) images were obtained with a Cambridge Stereoscan 240, and X-ray photoelectron spectroscopy (XPS) experiments were performed using a VG Scientific ESCA Lab 250 XPS spectrometer coupled with a monochromatic Al Kα source with charge compensation.

2.2. Preparation of sensor probe materials

Graphene oxide (GO) was synthesized from graphite powder according to modified Hummer's method (Zheng et al., 2013). Typically, graphite powder (5.0 g) and sodium nitrate (2.5 g) were blended in 120.0 ml of concentrated sulfuric acid (95%) and stirred for 30 min in an ice bath (≤ 0 °C). Then (15.0 g) of potassium permanganate was gently added in the prepared mixture solution, and the reactants were stirred out for a whole night at temperature < 20 °C. Afterwards, double-distilled water (150.0 ml) was added and the color of the mixture solution was transformed from dark greenish to brownish. The suspension was heated at 98 °C for one day with stirring to fully oxidize graphite. After cooling, hydrogen peroxide (30%) was added to the mixture, and the mixture was washed out several times with diluted HCl (5%) solution followed by washing with water. Finally, the product was filtered and dried under vacuum. In addition, AuNPs were prepared separately according to the previously reported protocol (Chandra et al., 2011). Briefly, 50 mL of 0.01 wt % $HAuCl_4$ in double-distilled H_2O was mixed with 1 ml of 38.8 mM trisodium citrate. After 1 min, 0.5 ml of a freshly prepared $NaBH_4$ solution was slowly added to the mixture. During the addition of $NaBH_4$, the color of the resulting solution changed from yellow to pink-violet, indicating the formation of AuNPs. Thereafter, Graphene oxide 0.5 mg/ml and AuNPs (1:1) were dispersed by ultrasonic agitation for 1hr to give a homogeneous suspension.

Prior to modifications, the bare GC electrode was sequentially hand polished with alumina powder (0.3, and 0.05 μm) on a wet soft polishing cloth, and then washed ultrasonically in ethanol followed by ultra-pure water for a few minutes to remove the adsorbed residual alumina particles. The cleaned electrode was dried and a mirror-like surface was obtained. To deposit a nanocomposite film on the electrode surface, a certain amount (5 μl) of the GO/AuNPs was deposited by casting the suspension onto a cleaned GCE surface and dried at room temperature. Further modification was carried out by the electropolymerization reaction in phosphate buffer solution (pH = 7.4) containing 1.0 mM DAN. The polymer films were formed by the potential cycles five times between 0.0 and +0.8 V at the scan rate of 100 mV/s. Then, the prepared electrode was rinsed with distilled water to remove loosely adsorbed GO/AuNPs/pDAN. Subsequently, the modified electrode was dipped in the EDC/NHS solution containing EDTA (10 mM) for 12 h for activation of carboxylic acid groups of EDTA