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An electrochemical immunosensor based on poly *p*-phenylenediamine and graphene nanocomposite for detection of neuron-specific enolase via electrochemically amplified detection

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ABSTRACT

In this work, a label-free electrochemical immunosensor was constructed on the base of poly p-phenylenediamine (PPD) and GR nanocomposite (PPD-GR). Screen-printed electrodes modified with PPD-GR nanocomposite and applied to advance enzyme-free and label free electrochemical immunosensor for detection of protein biomarker neuron-specific enolase (NSE). It was found that the PPD-GR nanocomposite exhibits excellent electrocatalytic activity towards ascorbic acid (AA) oxidation as analytical signal based on EC' mechanism. Due to the excellent electrocatalytic activity of PPD-GR nanocomposite, determination of NSE antigen was based on its obstruction to the electrocatalytic oxidation of AA after binding to the surface of electrode through interaction with the anti-NSE. The proposed immunosensor exhibited a wide linear range of 1.0–1000 ng mL^{-1}, with a low detection limit of 0.3 ng mL^{-1}. Furthermore, the proposed immunosensor were successfully used for the determination of NSE antigen in human serum samples.

Introduction

Different kinds of cancer are the result of unregulated growth of cells, and treating them can lead to more satisfactory results if the condition is diagnosed and the treatment starts early [1,2]. Lung cancer is among the deadliest cancers, the major causes of which include first and second smoking of tobacco, as well as industrial emission of substances such as arsenic, radon, and asbestos [3]. Since the concentration of biomarkers in human fluids such as blood and urine and other tissues is very low, a new sensitive and rapid method for detection of biomarkers such as neuron-specific enolase (NSE) is very important because it helps in detecting cancer at early stages, assessing tumor burden, monitoring disease progression and therapeutic treatment efficacy to improve long term survival of cancer patients [4–6]. Some methods are already used for the analysis of traces of biomarkers in blood [7,8]. Electrochemical methods, on the other hand, are widely used in various fields [9–14]. Electrochemical immunosensors compared with the conventional methods such as chemoluminescence immunoassay [15], enzyme-linked immunosorbent assay (ELISA) [16], radio immunoassay [17], and fluorescence immunoassay method [18], has gained wide attentions in the field of clinical analysis due to the advantages of easy-to-operate, rapid detection, low reagent consumption, easy miniaturization, high sensitivity and selectivity, and relatively inexpensive [19–21].

Label-free electrochemical immunosensors, offer numerous advantages including ease of fabrication, fast response, reasonable cost, specific responses and the ability to directly monitor the changes in the signals caused by the specific antibody/antigene interactions without the need for any labels [22–24]. Consequently, the devices have attracted a great deal of interest. Further, designing electrochemical biosensors based on immobilizing electro-catalytic on the surface of electrodes has been found to enhance the sensitivity of behaviors of unmodified electrode [25–27]. Among these materials, conductive polymers enjoyed significant recent attention due to their unique electrical, thermal, and mechanical properties [28–30]. There has been great interest in the fabrication of polymer-coated nanomaterials with unique and tailored properties for various applications. Different approaches have been applied to enhance the electrochemical behaviors of conductive polymers, as a means for improving the electrochemical performance of the resulting sensors. These include employing carbon-

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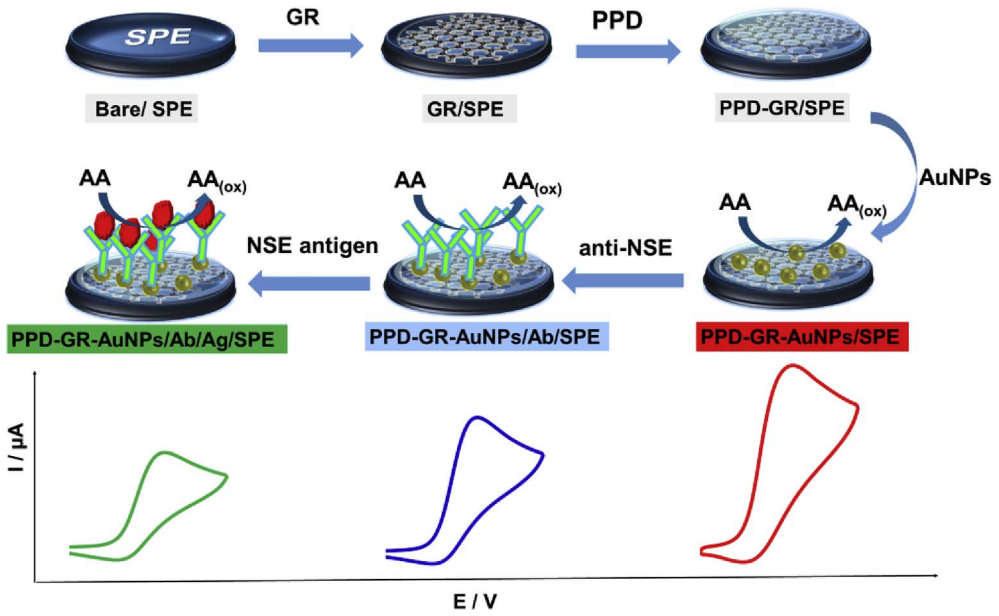

Scheme 1. Schematic exhibition of fabrication process of the immunosensor.

based materials as the support matrix. These composite nanomaterials provide a robust platform for sensing application and diverse functionalities from the polymer into a single particle.

A great deal of interest has been directed towards the application of graphene in different areas ranging from capacitors, electronic devices, composite materials and sensors [31–34]. The fabrication of electrochemical sensors based on graphene – conductive polymers has become a popular approach in many electroanalytical studies with good potential for constructing sensors with high sensitivity and selectivity to detect target molecules based on different analytical strategies [35–37].

Here, we report developing a sensing platform based on poly p-phenylenediamine (PPD) and GR nanocomposite (PPD-GR) through electropolymerization. This was next used in developing enzyme- and label-free electrochemical immune-sensor for a protein biomarker namely neuron-specific enolase (NSE). The study was mainly focused on using an EC' mechanism for the determination of NSE based on its obstruction to the electrocatalytic oxidation of ascorbic acid (AA) after binding to the surface of electrode through interaction with the anti-NSE. The results showed that the PPD-GR nanocomposite leads to an outstanding electro-catalytic activity towards oxidation as analytical signal based on EC' mechanism. Hence NSE could be successfully analyzed. Given the sensitivity, ease, reliability and specific nature of the method, it has the potentials for customization for the analysis of further biomarkers.

Experimental

Chemicals and apparatus

Except for the cases stated in the text, all materials were used without any further treatments. Pro-analysis grade graphite powder, ascorbic acid, phosphate salt, para-phenylenediamine (PD), hydrochloric acid, sodium hydroxide, as well as the solvents and reagents were obtained from Merck. Reagent grade chlorauric acid ($HAuCl_4$), 6-Mercapto-1-hexanol (MCH), cobalt dichloride ($CoCl_2{\cdot}6H_2O$) and thiourea were obtained from Sigma The anti-NSE antibody (Ab) and NSE protein (Ag) were from Abcam (Cambridge, UK).

An Autolab PGSTAT-302 N potentio/galvanostat was used for collecting the electrochemical data. The electrodes used in the electrochemical system, were a graphite screen-printed electrodes (SPEs) with a graphite working electrode, a graphite counter electrode and a silver pseudo reference electrode (Palm Instruments BV, Netherlands). The infrared (IR) spectra were recorded using a Shimadzu 8300 FT–IR instrument, and the morphology of nano-materials was studied using a Hitachi S-4160 scanning electron microscope (SEM). An electrochemiluminescence device Elecsys 2010 (manufactured by HITACHI Co.; Japan and Roche Co., Germany) was used as the referenced method.

Preparation of electrochemical immunosensor

The optimal conditions for preparing the immunosensors were determined. First, 2 mg of GR nanosheets obtained was dispersed in 1.0 mL ethanol by sonication for 1 h. 5 µL of GR nanocomposite suspension was coated onto the screen-printed working electrode surface and dried at room temperature to form a GR film at the SPE surface and prepare a GR–modified SPE (GR/SPE). The electropolymerization was performed using a solution of 5.0 mM PD on a GR-modified SPE (GR/SPE) with an applied potential ranging from −0.2 to 0.7 V at a scan rate of 50 mV s^{-1} for 9 cycles. Next, the electro-polymerized electrode was repeatedly rinsed with double-distilled water (ddH_2O) to form a poly para-phenylenediamine (PPD) film at the GR/SPE surface and prepare a PPD–modified GR/SPE (PPD-GR/SPE). Finally, the formation of AuNPs on the PPD-GR/SPE was carried out by CV scanning between the range potential of −200 and +1500 mV in a 0.5 mM $HAuCl_4$ solution containing sulfuric acid 0.5 M at scan rate of 60 mV s^{-1} for 6 cycles. The obtained electrode was marked as PPD-GR-AuNPs/SPE. After, the electrodes were rinsed with deionized water.

After that, 8 µL of 4 µg mL^{-1} Anti-NSE was placed on the PPD-GR-AuNPs/SPE for 18 h at 4 °C and then washed with PBS buffer (pH = 7.4) to remove unspecific physically adsorption. Following incubation, 8 µL of 2.0 mM 6-Mercapto-1-hexanol (MCH) was added onto the electrode for 30 min to blocked remaining non-specific active sites on working electrode. The as-prepared immunosensor (designed as PPD-GR-AuNPs/Ab/SPE) was used for detection of NSE biomarker during the experiment. Eventually the resulting device was stored at 4 °C. The overall fabrication process of preparing the immunosensor has been shown in Scheme 1.